MARINE BIOTECHNOLOGY IN THE TWENTY-FIRST CENTURY

PROBLEMS, PROMISE, AND PRODUCTS

NATIONAL RESEARCH COUNCIL

VISIT…

MARINE BIOTECHNOLOGY IN THE TWENTY-FIRST CENTURY

PROBLEMS, PROMISE, AND PRODUCTS

Committee on Marine Biotechnology:
Biomedical Applications of Marine Natural Products
Ocean Studies Board
Board on Life Sciences
Division on Earth and Life Studies
National Research Council

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Preface

In these proceedings the Ocean Studies Board and the Board on Life Sciences *ad hoc* Committee on Marine Biotechnology summarize and integrate information obtained from two workshops on Marine Biotechnology (October 5-6, 1999, and November 5-6, 2001). We use that information as a basis for recommending promising research areas in marine biotechnology. The 1999 workshop and its subsequent report emphasized environmental applications for marine biotechnology and included the topics of biomaterials, bioremediation, restoration, prediction and monitoring, and economic and regulatory aspects. The 2001 workshop (whose proceedings are incorporated into this report) emphasized biomedical applications of marine biotechnology and included the topics of drug discovery and development; genomic and proteomic applications for marine bioproduct discovery; biomaterials and bioengineering; and public policy, partnerships, and outreach. Considering marine biotechnology within this broad context, the committee identifies promising research areas and highlights issues that are slowing the implementation of marine biotechnology in the environmental and biomedical arenas. While aquaculture practices are relevant to the production and sustainability of marine natural products development, an in-depth examination of this large topic was beyond the scope of the current project.

The Committee acknowledges the contributions of its sponsors: the National Oceanographic and Atmospheric Administration's National Sea Grant College Program, the National Science Foundation, The Whitaker

Foundation, the Minerals Management Service, the Electric Power Research Institute, and the National Academy of Sciences. This report was also greatly enhanced by the participants of the two workshops. Those who participated in the 1999 workshop are acknowledged in its report. Here the committee acknowledges the efforts of those who gave oral presentations at the 2001 workshop: Rita Colwell, National Science Foundation; William Fenical, Scripps Institution of Oceanography; Guy Carter, Wyeth Ayerst; Mary Ann Jordan, University of California, Santa Barbara; Patrick Walsh, Rosenstiel School of Marine and Atmospheric Sciences; Bradley Moore, University of Arizona; Claire Fraser, The Institute for Genomic Research; Stephen Giovannoni, Oregon State University; Scott Peterson, The Institute for Genomic Research; Daniel Drell, U.S. Department of Energy; Anne Meyer, State University of New York at Buffalo; Rodney White, University of California, Los Angeles Medical Center; Cato Laurencin, Drexel University; Andrew Bruckner, National Oceanic and Atmospheric Administration; Joshua Rosenthal, National Institutes of Health; Donald Gerhart, University of Oregon; and James Cato, University of Florida Sea Grant. These speakers helped to set the stage for the fruitful committee discussions that followed the workshop.

In its discussions, the committee also relied heavily on the published proceedings of the 1999 workshop (NRC, 2000) and on oral summary briefs presented to the committee by 1999 workshop participants Laurie Richardson and Roger Prince (a committee member). The committee is also grateful to the following people who have provided other important material for consideration: Christine Benedict, Niels Lindquist, Robert Jacobs, and Eric Mathur. Ruth Crossgrove (NRC) provided assistance with editing.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report: Russell Kerr, Florida Atlantic University; Judith McDowell, Woods Hole Oceanographic Institution; David Newman, National Institutes of Health National Cancer Institute; Laurie Richardson,

Florida International University; Norman Wainwright, Marine Biological Laboratory; and Herbert Waite, University of California, Santa Barbara.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by John Burris, Beloit College. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Nancy Targett
Chair

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Executive Summary

Dramatic developments in understanding the fundamental underpinnings of life have provided exciting opportunities to make marine bioproducts an important part of the U.S. economy. Several marine based pharmaceuticals are under active commercial development, ecosystem health is high on the public's list of concerns, and aquaculture is providing an ever greater proportion of the seafood on our tables. Nevertheless, marine biotechnology has not yet caught the public's, or investors', attention. Two workshops, held in 1999 and 2001 at the National Academy of Sciences, were successful in highlighting new developments and opportunities in environmental and biomedical applications of marine biotechnology, and also in identifying factors that are impeding commercial exploitation of these products.

The following recommendations, based in large part on the workshop discussions, aim to identify the barriers restricting progress in the application of marine biotechnology to biomedicine and environmental science.

- The search for new drugs and agrichemical compounds should be revitalized by using innovative methods to gain a more fundamental understanding of the biosynthetic capabilities of marine organisms. Priority should be given to currently uncultured microorganisms including an increased effort in both culturing methods and culture-independent gene product analysis; exploration of unexamined habitats for new marine organisms; application of tools such as genome sequencing, functional

genomics, and proteomics to new "model" species of marine origin; and application of molecular biology to the synthesis of novel marine bioproducts. Use of these technologies should also foster sustainability and provide alternatives to the continued harvest of marine organisms.

- New paradigms should be developed for detecting marine natural products and biomaterials as potential pharmaceuticals, biopolymers, and biocatalysts, and for understanding how they exert their biological properties. Updated high throughput methods will need to be developed, adapted, and used to ensure that the testing is done in a timely fashion. In order to maximize the potential for commercial application, new strategies, such as DNA microarrays, mechanism-based profiling screens, integrated pharmacology, and increasingly sophisticated chemical ecology studies are needed for rapidly determining the mechanisms of action of new marine bioproducts. Access to updated and expanded biomedical screening programs is needed in a variety of therapeutic areas, involving broadly coordinated groups of investigators and novel strategies for the rapid identification of chemicals of biomedical importance.
- Better tools should be developed for using marine biotechnology to help solve environmental problems such as biofouling, pollution, ecosystem degradation, and hazards to human health.
- Greater emphasis should be given to research efforts that seek to commercialize marine bioproducts and assays for medical and environmental applications. Bringing these advances to commercialization will require stronger partnerships between scientists, the public, and innovative small companies. Fostering such partnerships, facilitating technology transfer, and streamlining government regulatory requirements will be needed for marine biotechnology to achieve its full potential.

Biomedical Applications of Marine Natural Products: Overview of the 2001 Workshop

INTRODUCTION

Marine biotechnology has demonstrated its potential across a broad spectrum of applications that range from biomedicine to the environment. Nevertheless, despite noteworthy successes (Tables 1–3) and the inherent promise of the ocean's vast biological and chemical diversity, marine biotechnology has not yet matured into an economically significant field. Fundamental knowledge is lacking in areas that are pivotal to the commercialization of biomedical products and to the commercial application of biotechnology to solve marine environmental problems, such as pollution, ecosystem disease, and harmful algal blooms.

To identify hurdles that are slowing the implementation of marine biotechnology within the biomedical and environmental sciences, the Ocean Studies Board (OSB) and the Board on Life Sciences (BLS) of the National Research Council (NRC) convened two workshops on marine biotechnology. One examined issues limiting the application of biotechnology to marine environmental science (October 1999; National Research Council, 2000), and the other examined issues surrounding biomedical benefits from marine natural products (November 2001).

In this report, the OSB and BLS *ad hoc* Committee on Marine Biotechnology summarize and integrate information obtained from the two workshops and highlight areas where new investments are likely to pay the

highest dividends in fostering the implementation of marine biotechnology in the environmental and biomedical arenas.

DRUG DISCOVERY AND DEVELOPMENT

The U.S. public is aware of the societal benefit of effective drug therapy to treat human diseases and expects that treatment will improve and become ever more accessible to the nation's population. This expectation is predicated on a continued and determined effort by academic scientists, government researchers, and private industry to discover new and improved drug therapies. Natural products have had a crucial role in identifying novel chemical entities with useful drug properties (Newman et al., 2000). The marine environment, with its enormous wealth of biological and chemical diversity (Fuhrman et al., 1995; Field et al., 1997; Rossbach and Kniewald, 1997), represents a treasure trove of useful materials awaiting discovery. Indeed, a number of clinically useful drugs, investigational drug candidates, and pharmacological tools have already resulted from marine-product discovery programs (Table 1). However, a number of key areas for future investigation are anticipated to increase the application and yield of useful marine bioproducts (see Fenical, p. 45 in this report). The broad areas where advances could have substantial impact on drug discovery and development are (1) accessing new sources of marine bioproducts, (2) meeting the supply needs of the drug discovery and development process, (3) improving paradigms for the screening and discovery of useful marine bioproducts, (4) expanding knowledge of the biological mechanisms of action of marine bioproducts and toxins, and (5) streamlining the regulatory process associated with marine bioproduct development.

New Bioproduct Discovery and Supply

The ocean is a rich source of biological and chemical diversity. It covers more than 70% of the earth's surface and contains more than 300,000 described species of plants and animals. A relatively small number of marine plants, animals, and microbes have already yielded more than 12,000 novel chemicals (Faulkner, 2001).

Unexamined habitats must be explored to discover new species. Most of the environments explored for organisms with novel chemicals have been accessible by SCUBA (i.e., to 40 meters). Although some novel chemicals have been identified at high latitudes, such as the fjords of British Colum-

TABLE 1 Some Examples of Commercially Available Marine Bioproducts

Product	Application	Original Source
Pharmaceuticals		
Ara-A (acyclovir)	Antiviral drug (herpes infections)	Marine sponge, *Cryptotethya cryta*
Ara-C (cytosar-U, cytarabine)	Anticancer drug (leukemia and non-Hodgkin's lymphoma)	Marine sponge, *Cryptotethya cryta*
Molecular Probes		
Okadaic acid	Phosphatase inhibitor	Dinoflagellate
Manoalide	Phospholipase A_2 inhibitor	Marine sponge, *Luffariella variabilis*
Aequorin	Bioluminescent calcium indicator	Bioluminescent jellyfish, *Aequora victoria*
Green fluorescent protein (GFP)	Reporter gene	Bioluminescent jellyfish, *Aequora victoria*
Enzymes		
Vent and Deep Vent DNA polymerase (New England BioLabs)	Polymerase chain reaction enzyme	Deep-sea hydrothermal vent bacterium
Nutritional Supplements		
Formulaid (Martek Biosciences)	Fatty acids used as additive in infant formula nutritional supplement	Marine microalga
Pigment		
Phycoerythrin	Conjugated antibodies used in ELISAs and flow cytometry	Red algae
Cosmetic additives		
Resilience (Estée Lauder)	"Marine extract" additive	Caribbean gorgonian, *Pseudopterogorgia elisabethae*

SOURCE: Adapted from Pomponi (1999).

bia and under the Antarctic ice, the primary focus of marine biodiversity prospecting has been the tropics. Tropical seas are well-known to be areas of high biological diversity and, therefore, logical sites of high chemical diversity. Much of the deep sea is yet to be explored, and very little exploration has occurred at higher latitudes. With rare exceptions (e.g., the analysis of deep-sea cores to identify unusual microbes), marine organisms from the deep-sea floor, mid-water habitats, and high-latitude marine environments and most of the sea surface itself have not been studied. The reason for this deficiency is primarily financial: oceanographic expeditions are expensive, and neither federal nor pharmaceutical-industry funding has been available to support oceanographic exploration and discovery of novel marine resources. The potential for discovery of novel bioproducts from yet-to-be discovered species of marine macroorganisms and microorganisms (including symbionts) is high (see Carter, p. 47 in this report; de Vries and Beart, 1995; Cragg and Newman, 2000; Mayer and Lehmann, 2001).

To optimize identification of marine resources with medicinal potential, the best tools for discovery must be used at all stages of exploration: in new locations, for collection of organisms never before sampled, and for the identification of chemicals with pharmaceutical potential. Increased sophistication in the tools available to explore the deep sea has expanded the habitats that can be sampled and has greatly improved the opportunities for discovery of new species and the chemical compounds that they produce. New and improved vehicles are being developed to take us farther and deeper in the ocean. These platforms need to be equipped with even more sophisticated and sensitive instruments to identify an organism as new, to assess its potential for novel chemical constituents, and if possible, to nondestructively remove a sample of the organism. Tools and sensors that have been developed for space exploration and diagnostic medicine need to be applied to the discovery of new marine resources.

Perhaps the greatest untapped source of novel bioproducts is marine microorganisms (see Fenical, p. 45 in this report; Bentley, 1997; Gerwick and Sitachitta, 2000; Gerwick et al., 2001). Although new technologies are rapidly expanding our knowledge of the microbial world, research to date suggests that less than 1% of the total marine microbial species diversity can be cultured with commonly used methods (see Giovannoni, p. 65 in this report). That means chemicals produced by as many as 99 percent of the microorganisms in the ocean have not yet been studied for potential commercial applications. These organisms constitute an enormous un-

tapped resource and opportunity for discovery of new bioproducts with applications in medicine, industry, and agriculture. Developing creative solutions for the identification, culture, and analysis of uncultured marine microorganisms is a critical need.

With the enormous potential for discovery, development, and marketing of novel marine bioproducts comes the obligation to develop methods for supplying these products without disrupting the ecosystem or depleting the resource. Supply is a major limitation in the development of marine bioproducts (Cragg et al., 1993; Clark, 1996; Turner, 1996; Cragg, 1998). In general, the natural abundance of the source organisms will not support development based on wild harvest. Unless there is a feasible alternative to harvesting, promising bioproducts will remain undeveloped. Some options for sustainable use of marine resources are chemical synthesis, aquaculture of the source organism, cell culture of the macroorganism or microorganism source, and molecular cloning and biosynthesis in a surrogate organism. Each of these options has advantages and limitations; not all methods will be applicable to supply every marine bioproduct, and most of the methods are still in development. Understanding the fundamental biochemical pathways by which bioproducts are synthesized is key to most of these techniques.

Molecular approaches offer particularly promising alternatives not only to the supply of known natural products (e.g., through the identification, isolation, cloning, and heterologous expression of genes involved in the production of the chemicals) but also to the discovery of novel sources of molecular diversity (e.g., through the identification of genes and biosynthetic pathways from uncultured microorganisms) (Bull et al., 2000). Manipulation of heterologously expressed secondary metabolite biosynthetic genes to produce novel compounds having potential pharmaceutical utility is at the forefront of current scientific achievements and has tremendous potential for creation of novel chemical entities (see Moore, p. 61 in this report; Khosla et al., 1999; Du and Shen, 2001; Floss, 2001; Rohlin et al., 2001; Staunton and Wilkinson, 2001; Xue and Sherman, 2001). In approaches parallel to those used for terrestrial soils, efforts need to be made to clone useful secondary metabolite biosynthetic pathways from natural assemblages of marine microorganisms (e.g., "cloning of the ocean's metagenome"). Use of these approaches to provide solutions to natural-product supply and resupply problems should be increased.

Screening for Bioactivity

Screening of natural materials for biologically active compounds has undergone radical changes over the past decade. With the advent of high-throughput-screening (HTS) technologies, an enormous number of materials, over 600,000, can be screened for a particular biological or biochemical property in a relatively short time, 2 to 4 months (Landro et al., 2000; Engels and Venkatarangan, 2001; Manly et al., 2001). Hence, a screen for a given disease target may be in operation for 3 months, during which time, marine natural products will be competing with large libraries of synthetic chemicals. New strategies for handling natural-product "mixtures" must be developed to synchronize with the accelerated HTS timetables. Marine natural-product mixtures, or extracts, must be purified and their active components rapidly identified. Development of technology to allow the prefractionation of crude extract materials prior to biological assay may allow for the rapid examination of active compound structures.

Another arena for improvement is the efficient elucidation of known and new natural-product structures. Hybrid analytical techniques that combine high-performance liquid chromatography (HPLC) with mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are becoming more common and accessible to natural-products chemists, and use of such techniques will expand in a variety of scholarly settings (Peng, 2000; Wilson, 2000). Continuous technological advances are needed in analytical chemistry associated with marine drug discovery to keep pace with comparable advances in biological screening of natural materials.

Currently, investigators do not have access to a broad range of biological assays for marine bioproduct discovery. Innovative strategies are needed that link groups of investigators to efficient drug-discovery programs. Such partnerships are envisioned for broad evaluations of new marine biomaterials in assays targeting a more complete range of human diseases (e.g., infectious, cardiovascular, cancer, neurodegenerative diseases, allergy and inflammation, and other metabolic disorders) as well as agricultural and veterinary needs. The increased number of discoveries of biomaterials possible through these partnerships and a corresponding improvement in the sophistication of their handling and distribution will encourage greater industrial evaluation of novel marine bioproducts.

Understanding Mechanisms of Action

The clinical and commercial development of many marine natural products languishes because of insufficient knowledge of how the compounds function in biological systems (Faulkner, 2000). It is precisely this understanding of pharmacological mechanism of action that has driven the development of such well-known pharmaceuticals as the potent anticancer metabolite paclitaxol (Taxol) from the Pacific yew tree (see Jordan and Wilson, p. 52 in this report; Correia and Lobert, 2001). Strategies that might be used in accelerating the development of marine biomaterials include focused mechanism-of-action studies, screening of libraries of purified marine metabolites by mechanism-based high-throughput assays, and characterization of a compound's biological effect using functional genomic and proteomic approaches. At the same time, it is crucial to make advances in integrated pharmacology to understand the effects of new and experimental drug therapies at the molecular, cellular, organ, and whole-animal levels. Molecularly based chemical ecological studies are a complementary approach to learn how marine biomaterials exert their properties in nature. In general, a greater emphasis on studying the mechanisms by which marine metabolites exert their potentially valuable properties will translate into an increased number of clinical candidates entering the development pipeline.

Marine organisms have demonstrated their utility as models to understand disease processes in humans (Table 1) (see Walsh, p. 57 in this report). Priority should be given to the identification and development of new model marine organisms to (1) identify novel targets for disease therapy, (2) discover novel chemicals for drug development, and (3) provide alternatives to current animal (and human) testing of drugs. With more complete genome sequences available from novel organisms, it will be more likely that an analog to human mutations can be found in a convenient test organism. Of critical importance in the development of new models is the availability of genome sequences from marine organisms. Genomic approaches, including whole-genome studies of appropriate model organisms, will accelerate discovery of new targets and new marine-derived drugs.

Recommendations for Enhancing Drug Discovery with Marine Biotechnology

- Explore new habitats.
- Develop tools to discover new resources.
- Discover and culture new marine microorganisms (including symbionts).
- Provide sufficient supply of bioproducts.
- Develop new screening strategies.
- Pursue strategies to hasten the discovery of new materials.
- Combine resources of academic, governmental, and industrial laboratories to expand access to biological screens in a variety of therapeutic areas.
- Expand research on pharmacological mechanisms.
- Establish new marine model organisms.
- Expand research on marine bioproduct biosynthesis and molecular biology.

GENOMICS AND PROTEOMICS APPLICATIONS FOR MARINE BIOTECHNOLOGY

Genomics

Genomics is the sequencing, annotating, and interpreting of information contained within the genome of an organism. Genome sequences of microorganisms represent the majority of the earliest work in genomics (Fraser et al., 2000a,b; Nelson et al., 2000) and have led to a better understanding of the biology of the organisms sequenced (Nierman et al., 2000). Microorganisms have been the focus of genomic research, probably because they have smaller genomes and therefore represent a more manageable sequencing goal. Recent technological breakthroughs in automated DNA sequencing and computational power have made it possible to rapidly sequence and annotate even large or complex genomes (Nelson et al., 1999; Heidelberg et al., 2000). Representations of the entire metabolic potential of microorganisms derived from the application of bioinformatics have indicated the presence of hitherto unsuspected metabolic pathways in even some very-well-characterized bacteria. Such genomic information provides a new basis for understanding physiological processes, such as responses of indicator species to environmental changes, stimuli that cause

an organism to synthesize a product of potential human benefit, or discovery of new gene targets for drug therapy, to name just a few (Read et al., 2001). The pharmaceutical industry has taken advantage of microbial genomics to search for novel vaccine targets in pathogenic microorganisms, greatly reducing the time and cost of drug target discovery (Pizza et al., 2000).

We have learned a tremendous amount during the infancy of the "genomic revolution." During this early period of genomic research, both basic and applied scientific questions have been addressed, and many have been answered. The ability to determine fully the genomic structure of an organism has allowed for finer resolution and greater speed in addressing specific biomedical questions, such as determining potential vaccine candidates from bacterial pathogens (Saunders et al., 2000). The genomic revolution has also led to the discovery of novel processes with major ecological implications, such as a rhodopsin-driven proton pump in an abundant but uncultured proteobacterium from the ocean's surface. This discovery—based on the application of genomics to analyses of easily collected but uncultured marine microorganisms—has opened a new path to understanding of light-harvesting and near-surface open-ocean primary productivity (Béjà et al., 2000, 2001).

Current genomic methods enable researchers greater speed, sensitivity, and resolution over other commonly used molecular methods. As the science of genomics continues to mature, new technologies will emerge. Their implementation and integration with other technologies will be essential for advancement in the marine biomedical and environmental sciences (Cary and Chisholm, 2000).

With recent decreases in sequencing costs and increases in the number of high throughput sequencing facilities at private, governmental, and nonprofit laboratories in the United States, complete genome sequencing of many established and novel model organisms, including eukaryotic marine organisms, is realistically attainable (Fraser, p. 66 in this report). In addition, the development of genomic technologies, such as bacterial artificial chromosomes (BACs) enabling the cloning of large DNA fragments, and the expansion of computational tools for genomic analysis now allow the complete sequencing and genomic analysis of entire biological systems to be an achievable goal. Many marine eukaryotic organisms (e.g., corals, sponges, and tube worms) maintain large and diverse populations of microbial symbionts. The complete genome sequences of these consortia will not only lead to unprecedented understanding of the interactions be-

tween host and symbiont, but will also expedite the discovery of novel metabolites, such as drugs and fine chemicals, that are the products of such consortia.

As much as 40% of a genome encodes for genes whose functions remain unknown, highlighting genome sequencing and annotation as a parts list, but not the organism's instruction manual. These unknown gene functions represent a starting point for scientists studying either a specific organism or a biological relationship (e.g., host and symbiont). However, for complete genome sequences to be utilized by the greatest number of scientists possible, particular species or strains must be identified and carefully selected as models (see Walsh, p. 57 in this report). Genomic information should enable as large a scientific community as possible to expand its current research; the selection of an inappropriate organism will not allow for a broad application. Although the cost of sequencing has decreased, it is still important not to waste effort on redundant genomic projects. To reduce duplication of effort, the sequence data and the databases and tools that allow scientists to analyze and utilize the data must be maintained and made accessible. Additionally, projects that require sequencing of large genomes must be subjected to a careful cost and value analysis of finished genome versus draft sequencing (a less expensive approach, with missing genes and misassembled regions of the genome). The scientific community at large must take responsibility for many of these pragmatic considerations, selection of appropriate model species for sequencing, maintenance of publicly accessible databases, and determination of the relative value of finished genome versus draft sequencing.

Marine Microbes and Genomics

A large and interesting pool of potentially bioactive molecules is likely to be affiliated with the microbial population of the oceans (see Fenical, p. 45, and Giovannoni, p. 65 in this report). These populations are typically composed of a few cosmopolitan organisms, but the overall group diversity is very high. It has been a problem to bring many of these organisms into culture where they can be studied more easily. Currently, methods are being developed that have allowed several of these cosmopolitan marine bacteria to be cultured.

There are numerous other marine microorganisms that have not been cultured. Some of these bacteria might be culturable when more innovative approaches are developed (see Giovannoni, p. 65 in this report). How-

ever, it is unlikely the species diversity of the oceans will be brought completely into pure culture. As a more tractable alternative, genomic and bioinformatic methods are powerful new tools to access the gene products of these uncultured microorganisms. The total DNA from an environmental sample can be purified without first culturing the organisms (Ward et al., 1990, 1992; Rondon et al., 2000). This environmental DNA can be sequenced analogously to a genome and allows access not only to the protein products of uncultured bacterial species, but also to the genomic potential of the environment (or "ecological genomics"). The current technology is already in place for such survey sequencing of environmental DNA. Following bioinformatic analysis, cloning and expression of selected genes from the uncultured bacteria will likely lead to the discovery of novel bioactive molecules. These methods have been used successfully in looking for antimicrobial proteins from uncultured soil bacteria.

DNA Microarrays

Microarray technologies offer an additional tool for high-throughput analyses of the genome of an organism and the responses of an organism to specific changes. In an organismal DNA microarray, thousands of protein-encoding DNA sections are arrayed on a solid support structure (e.g., glass slide or nylon membrane). The array is then hybridized with a nucleic acid from a test sample, and the genes common to both the microarray and the test sample can be detected. As one example of an application of DNA microarray technology, the nucleic acid test sample can be the total messenger RNA (representing those genes that are likely being expressed as proteins) isolated before and after introduction of an environmental stress (e.g., addition of a pollutant, challenge with a bioactive molecule, and change in temperature). In this case, the genes that the organism differentially expresses as a result of the stress can be determined. Therefore, microarrays can be useful tools to examine gene expression patterns of a model organism in response to a variety of stimuli. That capability makes them powerful new diagnostic tools with applications in environmental monitoring, bioremediation, and drug discovery and reiterates the importance of careful selection by the scientific community of model organisms for complete genome sequencing. Obviously, this tool is most powerful for organisms for which the complete genome is sequenced, but even if expressed sequence tags (ESTs) are spotted on the microarray, experiments can yield very useful information (see Walsh, p. 57 in this report).

Microarray techniques also are powerful tools for examining the genomic differences between two organisms, particularly if a complete reference genome is available for comparisons. The total genomic DNA of the second organism is used as the test sample for hybridization to the genome microarray of the first organism. These data allow rapid determination of the genes found on the reference genome and genes shared between the two organisms. Such comparisons to reference genomes are very useful to identify genes that are distinctive to different individuals or strains from different environments. Medical microbiologists have taken advantage of such comparisons to find pathogenicity "islands" in disease-causing bacteria. By sequencing and building a genome array of a pathogenic bacterial strain and hybridizing the array with less pathogenic strains of the same species, genomic regions resulting in increased pathogenicity have been determined (see Fraser, p. 66 in this report). Analogously, the genes responsible for the production of bioactive molecules by marine eukaryotes or prokaryotes can be more quickly determined after the genome sequence of the model organism is determined and a complete genome array constructed.

As microarray methods become more common, duplication of effort and resources is more likely. Much of the cost of microarray technology is in the design and production of the test slide. If care is not taken, individual researchers might waste important time and effort producing duplicate microarrays for the same species. One way to reduce the risk of duplication is through centralization of a microarray production facility, either virtual or physical, for community-wide use. Such a facility may also help to standardize methods and allow comparisons of experiments conducted in different laboratories.

Proteomics

Proteomics is the characterization of the proteins specified by the genome of an organism. Proteomics is a new science that is considered to be an extension of the Human Genome Project, because it links genetics with physiology and provides clues not only to the function of genes encoding certain proteins but also to the function of the proteins. There are molecular techniques that allow determination of expressed proteins in a given system. However, these techniques are very time-consuming when used to identify the posttranslational modification of proteins. An understanding of the modification of proteins will become increasingly more important in the search for novel biomolecules.

The potent combination of classic microbiological techniques, proteomics, and genomics must be recognized. Lab culture of microorganisms, when linked with genomic analysis and the use of proteomics, represents a continuum of knowledge about the adaptations of microbes to their changing environments. Intersections of these three investigative paths may provide crucial information for identifying novel metabolites, pathogens, and for characterizing environmental remediation needs.

Unfortunately, proteomic methods are not yet high throughput and are fairly costly when considering analyses of an entire genome. As these technologies develop, especially at the national laboratories, it will be important for proteomics to be integrated into marine biomedical and environmental research programs.

Genomics and Proteomics as Exploration Science

Genomic studies are not always hypothesis driven; their fields are exploratory. The technology enables scientists to generate data from which hypotheses can be formulated and tested. This exploration activity should be considered an asset because of its potential to increase our knowledge base, and it should not be considered a liability, particularly in the review of proposals incorporating genomics and proteomics technologies. It is important to make certain, however, that genomic and proteomic data are publicly available, and in a useful form so that the data can be used for hypothesis-driven research. Therefore, it is important that genomic and proteomic databases be developed, maintained, and made available as research tools.

Recommendations to Enhance the Application of Genomics and Proteomics to Marine Biotechnology

- Incorporate genome sequencing, proteomics, and bioinformatics with nonculture-based methods to survey diverse marine environments and improve screening methods for uncultured microbes.
- Ensure that high-throughput sequencing and informatics facilities are available to the marine biotechnology research community.
- Develop a community-wide consensus on model organisms for genome sequencing, and develop both a priority list and a “wish” list.
- Develop arrays for determining differences among the genomes of different organisms.

- Develop whole-genome and EST arrays to determine gene-expression patterns of model organisms as rapid screens for bioactivity and drug discovery.
- Develop environmental genome microarray chips to identify function or coregulation of genes from the environment.
- Determine the potential usefulness of a centralized microarray facility to make reagents, develop and disseminate informatics tools, and provide training to the marine biotechnology community. Reduce redundant funding of array development and nonstandardized hybridization techniques that will prevent cross-experiment comparisons.
- Ensure that the "exploratory" data generated in both genome sequencing and functional genomic studies are available to expedite and enable hypothesis-driven science. Include the development and maintenance of useful public databases and improved training of the scientific community.

BIOMATERIALS AND BIOENGINEERING

Well beyond the obvious providers of food, the world's seas have always been bountiful providers of special materials valued for human health and pleasure. Access to this resource historically has been hindered by the apparent hostility of the seawater environment to manufactured materials and engineering concepts of *terra firma*. In spite of the extraordinary potential of the marine environment for new biomaterials, the environmental risks and exploration costs have been prohibitive.

In the past decade, new tools of biotechnology have been introduced that are producing extraordinary new products and assays based on the new understanding of genetic factors and their expression as complex biological molecules. Applying these tools to the marine environment provides opportunities to unlock similar micro-molecular vaults of marine biomedical products so that they can join other macro-biomaterials already harvested from the sea for thousands of years.

Novel Characteristics of Macro-Biomaterials from Marine Organisms

Marine biomaterials are a heterogeneous group of organic-, ceramic-, and polysaccharide-based polymers that hold promise for a variety of new approaches to the treatment of disease (see White and White, p. 79, and Laurencin, p. 83 in this report). The marine environment is home to

numerous microporous materials, such as those that provide the framework for coral reefs or those that compose the spines of sea urchins. These macrobiomaterials are characterized by highly interconnected porous networks, with a wide range of porosities (Weber and White, 1973). Because of their geometric and material properties, coral structures and urchin spines are used in vascular graft construction and orthopedic surgical repairs (see White and White, p. 79 in this report). Identification of the natural convoluted geometries and fouling-resistant surface features of coral has been a key factor prompting consideration of other biotechnology approaches to successful biomimicry and biomaterials manufacture. Marine organisms can provide many more novel models for biomolecular materials design.

New biotechnologies have been introduced for biocompatible, self-limiting, implantable biomedical devices based on "storage biopolymers," such as polyhydroxyalkanoates, which are abundant in marine microorganisms (see Laurencin, p. 83 in this report; Madison and Huisman, 1999). New opportunities also exist for high-value biomedical products, such as drug-delivery units, based on chitin from marine crabs and other crustaceans (Felt et al., 1998; Janes and Alonso, 2001; Sato et al., 2001). The enormous supply of chitin and chitosan biopolymers serves as a base for hydrogel-like hosts for various medicinal ingredients, including antibiotics, and provides good wound-dressing qualities for abrasions and ulcers. Work is under way to utilize novel combinations of storage biopolymers, particularly polyhydroxybutyrate, with coral segments to fabricate a scaffold that can be used in bone repair (Laurencin et al., 1996; Madihally and Matthew, 1999; Suh and Matthew, 2000).

Facilitating Work at Surfaces

Marine surfaces are important planes of research and exploration for biotechnological applications. Of particular interest are the characteristics of submerged natural surfaces that resist corrosion and adhesion and the opposing characteristics of selected organisms that allow them to adhere tightly to wet, slimy surfaces. The oceans' intrinsically nonstick, low-drag plant and animal surfaces and the adaptations of some species to adhere to wet surfaces hold incredible promise for future biomedical applications (Anderson, 1996). The most well-known example is perhaps the common blue mussel, *Mytilus edulis*, with its strong byssal threads, and adhesion discs which allow it to remain attached in very high energy environments, including pounding surf. However, to fully commercialize these character-

istics, critical issues of cross link biocatalysis and water displacing post-translational modifications of secreted adhesive biopolymers must be resolved (see Benedict, p. 69 in this report). In addition to the submerged biological and physical surfaces, the air-sea interface is important as a biomaterial source and model for bioengineering of new artificial lungs and biolubricants. The sea surface is ubiquitously coated with surface-active natural molecules that are the modulators of gas and particle exchange across the liquid-gas interface. Similar analogies exist between sea-surface films and natural biolubricants of human tear films in the blinking human eye.

Applications for Novel Marine Biomaterials

There are many areas in which a better understanding of physiological processes in marine organisms may improve the development of biomedical tools. For example, coral growth and healing may improve the understanding of bone development and healing. A better understanding of the principles of biomimicry of marine surfaces may allow the development of micro- and nano-structured implants for tissue regeneration. Sea-surface explorations should be a routine part of deep sea and coral examinations for materials with bioengineering and tissue-engineering applications. New photocatalytic materials will likely be found in the uppermost sea-surface zones otherwise neglected in explorations of deep sea and coral surfaces, as evidenced by the recent discoveries of light-driven photopigment reactions near the sea-air boundary (Béjà, et al., 2000, 2001).

Biotechnological tools may reveal how marine biocatalysis promotes secure underwater adhesion, with strength and security yet unmatched by terrestrial sources and synthetic approaches. Underwater self-cleaning, self-lubricating plant and animal surfaces may be better understood with new biotechnology, the results of which could be used for the benefit of dry eye and dry mouth sufferers and lubricant-depleted human tissues.

The sustained productivity and economic successes of collection and bioengineering of kelp and other macroalgal products into agars, alginates, and food products provide models for the future of marine biotechnology as it applies to marine biomaterials. Another goal is to identify and exploit the micro- and nano-scale novel characteristics of marine organisms that can make excellent templates for biomaterials and drug delivery of therapeutic devices with potential application in human medicine and bioengineering.

Recommendation for Enhancing Development of Marine Biomaterials

- Explore for new sources and characterize the novel physical and chemical characteristics of marine biomaterials for potential innovative biomedical and environmental engineering applications including biomolecular materials design.

PUBLIC POLICY, PARTNERSHIPS, AND OUTREACH IN MARINE BIOTECHNOLOGY

Although marine biotechnology has an expanding impact on biomedical, agrichemical, and environmental applications, important knowledge gaps still exist. More discussion among scientists, private businesses, legislators, and the public must be organized to ensure broader implementation and commercialization of products. These gaps include issues of intellectual property rights, mechanisms of technology transfer, knowledge of regulatory requirements (Gerhart, p. 94 in this report), resource sustainability (Bruckner, p. 87 in this report), and the importance of forging partnerships between and among the various constituent stakeholders (see Rosenthal, p. 91, and Cato and Seaman, p. 97 in this report). Businesses, legislators, and the public need to understand the importance and promise of ocean biodiversity as a source for marine biotechnological innovation and recognize the promise and problems of marine biotechnology as they specifically relate to environmental and biomedical applications.

Intellectual Property Rights and Technology Transfer

The commercial development of marine bioproducts is complex, time-consuming, expensive, and risky (see Gerhart, p. 94 in this report). Thus, protection of an individual's intellectual property rights through patents, copyrights, trade secrets, or trademarks for a potential product is essential for encouraging commercial development of that product (Smith and Parr, 1998). However, academic environments create special challenges for individual patent protection, primarily because academic culture is based on intellectual freedom, open discourse, and individual achievement. The role of the university is viewed as one of creating and disseminating knowledge, not withholding and protecting information. Indeed, most university research is externally funded, and investigators are expected to publish exten-

sively. Thus, a fundamental disconnect exists between the general view of the university's mandate for openness and access and the need for patent protection to ensure that products and ideas developed within an academic setting can be realistically available for the lengthy and expensive process of commercialization.

To facilitate the interaction of industry and academia, most universities now maintain offices that facilitate technology transfer. The concept of university-industry technology transfer is attributed to Vannevar Bush, science advisor to President Franklin Delano Roosevelt. Initially, the idea was driven by concerns about U.S. national security during World War II. In 1980, the Bayh-Dole Act modernized the concept and stimulated the creation of the university technology transfer programs as we know them today. This act mandates that university researchers must disclose inventions made with federal support and requires universities to report inventions to the U.S. government. According to the act, universities may elect to take title to an invention resulting from federally funded research but notes that if they do so, they must diligently pursue patenting and commercialization. Universities typically accomplish technology transfer through licensing (Abramson et al., 1997).

The Regulatory Process

Federal regulations control the development and marketing of bioproducts with human health and safety implications. Preclinical- and clinical-product development related to the regulatory process can take an average of 5 to 7 years and can cost from $15 million to more than $200 million (Cato, 1988; Trenter, 1999), with some reports of costs as high as $800 million (DiMasi, 2001). This cost can be one of the most important hurdles to surmount in the development of a marine-derived bioproduct. Mechanisms to streamline the process and lower the expense must be explored if marine bioproduct development for medical applications is to succeed.

A look at the marine bioproducts available today through the advances of marine biotechnology suggests that numerous products of marine origin have already been successful. Products have been brought to market (Tables 1 and 2), and ideas have been licensed for commercial development (Table 3). Despite these successes, there are concerns that the potential of many marine bioproducts is being compromised because the transition from labo-

TABLE 2 Some Commercially Available Marine-Derived Biomedical Research Probes

Source	Probe	Function	Price
Sponge	Manoalide	Phospholipase A2 inhibitor	$120/mg
	Calyculin A	Protein phosphatase inhibitor	$105/25 µg
	Luffariellolide	Phospholipase A2 inhibitor	$100/mg
	12-epi-scalaridial	Phospholipase A2 inhibitor	$136/mg
	Latrunculin B	Actin polymerization inhibitor	$90/mg
	Mycalolide B	Actin polymerization inhibitor	$212/20 µg
	Swinholide A	Actin microfilament disruptor	$100/20 µg
Dinoflagellate	Okadic acid	Protein phosphatase inhibitor	$75/25 µg
Bryozoan	Bryostatin 1	Protein kinase C activator	$88/10 µg
Sea hare	Dolastatin 15	Microtubule assembly inhibitor	$125/mg

SOURCE: BioMol [www.biomol.com].

ratory discovery to early commercial development has not been efficient or successful, and regulatory hurdles have not been surmounted. To overcome these bottlenecks it is necessary to educate marine scientists more aggressively about intellectual property rights and regulatory processes. That education should result in increased invention disclosure rates that will preserve nascent patent rights and ensure that more products are available for commercialization. Efforts should also be made to encourage transitional research, thus enhancing the movement of an idea to marketable product.

Sustaining Resources Through Diverse Partnerships

Because the continued successful development of marine biotechnology is intimately connected with ocean biodiversity, it is essential that efforts be made to ensure that biodiversity is protected. Tropical regions with especially rich biological marine ecosystems are often regions of intense poverty (see Bruckner, p. 87 in this report). Short-term, regional financial incentives, which seem to have an immediate impact on the poverty, must be balanced with the long-term sustainability of the resource. Partnerships must be developed to protect marine resources in tropical areas in particular, thus ensuring a positive economic outcome and the long-term protec-

TABLE 3 Marine-Derived Antitumor Compounds Licensed for Development

Marine Source	Drug	Organism	Current Status
Sponge	Discodermolide	*Discodermia dissoluta*	To enter Phase I trials in 2002; licensed to Novartis
	Isohomo-halichondrin B	*Lissodendoryx* sp.	Licensed to PharmaMar S.A.; in advanced preclinical trials
	Bengamide	*Jaspis* sp.	Synthetic derivative licensed to Novartis; in clinical trials
	Hemiasterlins A & B	*Cymbastella* sp.	Derivatives to enter clinical trials in 2002; licensed to Wyeth-Ayerst
	Girolline	*Pseudaxinyssa cantharella*	Licensed to Rhone Poulenc
Bryozoan	Bryostatin 1	*Bugula neritina*	In Phase I/II clinical trials in U.S./ Europe; U.S. National Cancer Institute (NCI) sponsored trials

tion of the resource (see Rosenthal, p. 91 in this report). In all cases, commercial development from natural populations of marine organisms must be sustainable if it is to make economic sense. Sustainability is one of the central challenges in further development of marine biotechnology, and it must be addressed before large-scale marine harvests can begin. Innovative approaches to partnerships between stakeholders can help to support access to marine resources and to ensure their development as sustainable assets. Agreements that include training and education of local populations can be particularly valuable for long-term resource sustainability.

TABLE 3 Continued

Marine Source	Drug	Organism	Current Status
Sea hare	Dolastatin 10	*Dolabella auricularia*	Phase I clinical trials in U.S.; NCI sponsored trials
Tunicate	Ecteinascidin 743	*Ecteinascidia turbinata*	Licensed to PharmaMar S.A.; in Phase III clinical trials in Europe and in U.S.
	Aplidine	*Aplidium albicans*	In Phase II clinical trials; licensed to PharmaMar S.A.
	Isogranulatimide	*Didemnum granulatum*	Licensed to Kinetik, Canada
Gastropod	Kahalalide F	*Elysia rubefescens*	In Phase I clinical trials; licensed to PharmaMar S.A.
Actinomycete	Thiocoraline	*Micromonospora marina*	Licensed to PharmaMar S.A.; in advanced preclinical trials

SOURCE: Data from David J. Newman, National Cancer Institute, Natural Products Branch, Frederick, Md.

Enhancing Public Awareness and Understanding of Marine Biotechnology

As marine biotechnology rapidly evolves, there is an increasing gap between use of technology and the public's understanding of that science and its implications. To avoid the public's misunderstandings that plague agricultural biotechnology (e.g., genetically modified foods), it is essential that scientists partner with the public to provide information that addresses both the promise and possible problems of marine biotechnology. A multi-tier approach should be developed that connects individuals from science,

education, business, and media to address the public's formal and informal educational needs (Cato and Seaman, p. 97 in this report).

For marine biotechnology, implementation of improved technology transfer, sustainable environmental stewardship, innovative partnerships, and enhanced public education should result in increased production of marine bioproducts and approved marine therapeutics, enhanced revenues from marine bioproducts, and positive impacts on coastal economic development.

Recommendations to Enhance Research and Development, Partnerships, and Outreach for Marine Biotechnology

- Aggressively educate marine scientists about intellectual property rights and regulatory processes to increase invention disclosure rates and preserve patent rights so that more products will be available for commercialization.
- Encourage academic rewards for transitional research between academic and industry scientists to facilitate the commercialization of marine bioproducts.
- Develop innovative approaches to partnerships between stakeholders to support access to ocean resources and to ensure their use as sustainable assets.
- Educate the public to the promise and problems of marine biotechnology to avoid fears rooted in misunderstanding and misconception.
- Enhance technology transfer services in universities.

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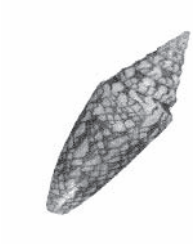

Environmental Aspects of Marine Biotechnology: Overview of the 1999 Workshop

INTRODUCTION

The U.S. coastal population is growing rapidly, so a healthy marine environment is ever more vital for the well-being of Americans. The 1999 workshop identified several areas where there are serious needs and exciting opportunities for biotechnology to improve our understanding of the marine environment to better ensure its protection (National Research Council, 2000). These areas include the bioremediation of oil and other spills, the health of coral reefs and other marine environments, and potential threats to human health caused by toxic blooms and microbial contamination. The intervening years have increased concern for the health of the marine environment, but they have also provided optimism that progress is possible. In particular, recent advances in microbiological and in toxicological testing discussed at the 2001 workshop could transform research in those areas and bring real improvements in our ability to understand and maintain the environment and protect human health.

BIOREMEDIATION

Large amounts of oil are extracted from the sea floor and even larger volumes are shipped. Unfortunately, remediation is sometimes required for accumulated daily small spills, and occasional major spills. Given the staggering scale of oil usage, which is estimated at 3.5 billion gallons ($1.2 \times$

10^{10} l) per day (Anonymous, 2001), even the spillage of a tiny fraction (estimated at <0.0035% in 1997 (Etkin, 1999)) means that more than 120 million gallons are spilled into the world's oceans each year. Fortunately, despite the increasing volume of transported oil, the amount spilled from catastrophic accidents has been generally decreasing (Etkin, 1999). Nevertheless, there is intense public pressure for the infrequent major spills to be cleaned up in an environmentally appropriate manner as quickly as possible. Bioremediation offers a clean-up technology that works to speed natural degradation processes (Lee and deMora, 1999), and it has already achieved notable success following the spill from the *Exxon Valdez* in Alaska (Prince and Bragg, 1997). Extension of this approach to marshes, harbors, and dredged sediment will further facilitate the remediation process.

Modern genomic and culturing tools, described at the second workshop, will revolutionize our understanding of, and hence our ability to manipulate and restore, marsh environments and dredged material. Both are fragile substrates where careless human intervention can cause more harm than good. Physical treatments of marshes can cause more damage than the initial spill (Canadian Coast Guard, 1995) and anaerobic dredge spoil may release heavy metals if allowed to become aerobic without appropriate containment (National Research Council, 1997). Bioremediation can potentially offer cost-effective and environmentally appropriate treatments for these troublesome situations. Sustained effort will be required in both basic science, to understand the microbial metabolic potential that we may be able to exploit, and in field applications. For example, recent mesocosm experiments (Dowty et al., 2001) and field studies at the San Jacinto test site in Texas (Simon et al., 1999) showed promising leads in developing bioremediation. Integrating the new genomics and proteomics tools and environmental remediation experiments, perhaps by encouraging collaborative research, is an obvious way of stimulating rapid progress in this important area of biotechnology. Reliable bioremediation strategies for marshes and dredged materials would be an important contribution toward maintaining ecosystem health in the face of continued exploitation of our coastline.

ENVIRONMENTAL HEALTH

The ever-growing coastal population is placing an increasing burden on the natural processes of the coastal environment. Fisheries are declining (Jackson et al., 2001), and there is increasing concern about sewage con-

tamination of recreational beaches (Griffin et al., 2001), fertilizer runoff (Frink et al., 1999), toxic dinoflagellates (Burkholder and Glasgow, 2001), and diseases of coral reefs (Richardson, 1998) and other organisms (Harvell et al., 1999). These are not minor concerns. Outbreaks of harmful algal blooms, such as the toxic *Pfiesteria* in North Carolina and then the Chesapeake Bay in the late 1990s, caused near hysteria in the press (Magnien, 2001). In spite of substantial efforts, controversy still exists over what causes these outbreaks, although excess inorganic nutrients in the water seem to play a role (National Science and Technology Council, 2000). Such outbreaks have substantial costs both to individuals, such as fishermen, and to communities (Anderson et al., 2000). Preventing these acute events in a cost-effective manner would have many benefits. If they cannot be totally prevented, it would be appropriate to develop control strategies to minimize their longevity. One possibility is that harmful algal blooms are controlled by trace elements in the water (Anderson and Garrison, 1997). Manipulating these elements might be a cost-effective tool, but this possibility needs additional investigation before it can be seriously addressed. Alternatively, it might be possible to biocontrol some organisms by adding specific pathogens when a bloom has become established. For example, a virus has been isolated that is apparently capable of controlling the brown-tide microalga *Aureococcus* (Milligan and Cosper, 1994).

Healthy marine environments, especially coral reefs, have substantial economic benefits for local communities (e.g., Hundloe, 1990). Tourism, possibly one of the world's largest industries, often relies on a healthy coral ecosystem to attract customers. Yet healthy marine environments seem to be declining around all our coasts. At times, this is clearly due to damage from development, perhaps because of increased nutrients or increased silt in the water. In other cases, the decline is attributed to disease, although very few of the diseases of marine organisms have been correlated with a specific pathogen (Richardson et al., 1997; Richardson, 1998; Harvell et al., 1999). Thus, at present it is difficult to be sure how widespread these diseases are, whether putative causative organisms are indeed responsible for disease, or whether the organisms exploit certain environmental conditions at different times or in different places.

Marine biotechnology, and particularly the development of new techniques in genomics and proteomics, offers the potential for exquisitely sensitive diagnostic tests to clearly identify the initial outbreaks of toxic organisms, discover their distribution in estuaries and oceans, and perhaps help to identify biocontrol agents. Similarly, these techniques could reliably

detect the causative agents of many of the diseases that are afflicting coral reefs and other environments (Figure 1). In turn, it might be possible to diagnose some of the conditions that predispose ecological systems to outbreaks of toxic organisms and diseases and thereby protect vital estuarine and marine resources.

There is also a need for active restoration of compromised ecosystems, such as degraded coral reefs. It may be necessary to raise the replacement organisms by aquaculture to provide the necessary material for restoration. Unfortunately, the few attempts at restoration that have been undertaken to date, such as the work with stony corals, have not been very successful. Reproduction and larval metamorphosis in many shellfish are controlled by specific environmental chemicals, such as prostaglandins. Sometimes inexpensive mimics can be used to trigger desired events (e.g., hydrogen peroxide as a replacement for specific prostaglandins). Perhaps these findings can be extended to other invertebrates, but development of techniques for large-scale production of diverse organisms for restoration will take a sustained and targeted research program.

HUMAN HEALTH

One of the primary concerns in public health is the risk that humans using the marine environment will encounter microbial pathogens, especially from human excreta (Griffin et al., 2001). Unfortunately, the diversity of potentially harmful microorganisms is so great that routine monitoring for pollution relies on the search for "indicator organisms." Historically, these indicators have been used because they are conservative, they occur with high concentrations of pathogens, and they cannot replicate in the environment (Griffin et al., 2001). All current tests involve culturing, including those for total and fecal coliforms, *Escherichia coli*, enterococci, and *Clostridium perfringens*. These have served humans well, but they are by no means perfect; tests take time to culture and analyze and do not assess indigenous nonsewage organisms, such as *Vibrio vulnificus*, which can cause severe disease and even death in immuno-compromised persons. Modern molecular tools offer the promise of essentially instant tests, perhaps akin to the antibody tests for common ailments now available in physician's offices. Alternatively, PCR-amplification tests could be developed for particularly troublesome organisms. Real-time tests would revolutionize health-risk monitoring of recreational beaches (Grimes, 1999; Rose and Grimes, 2001).

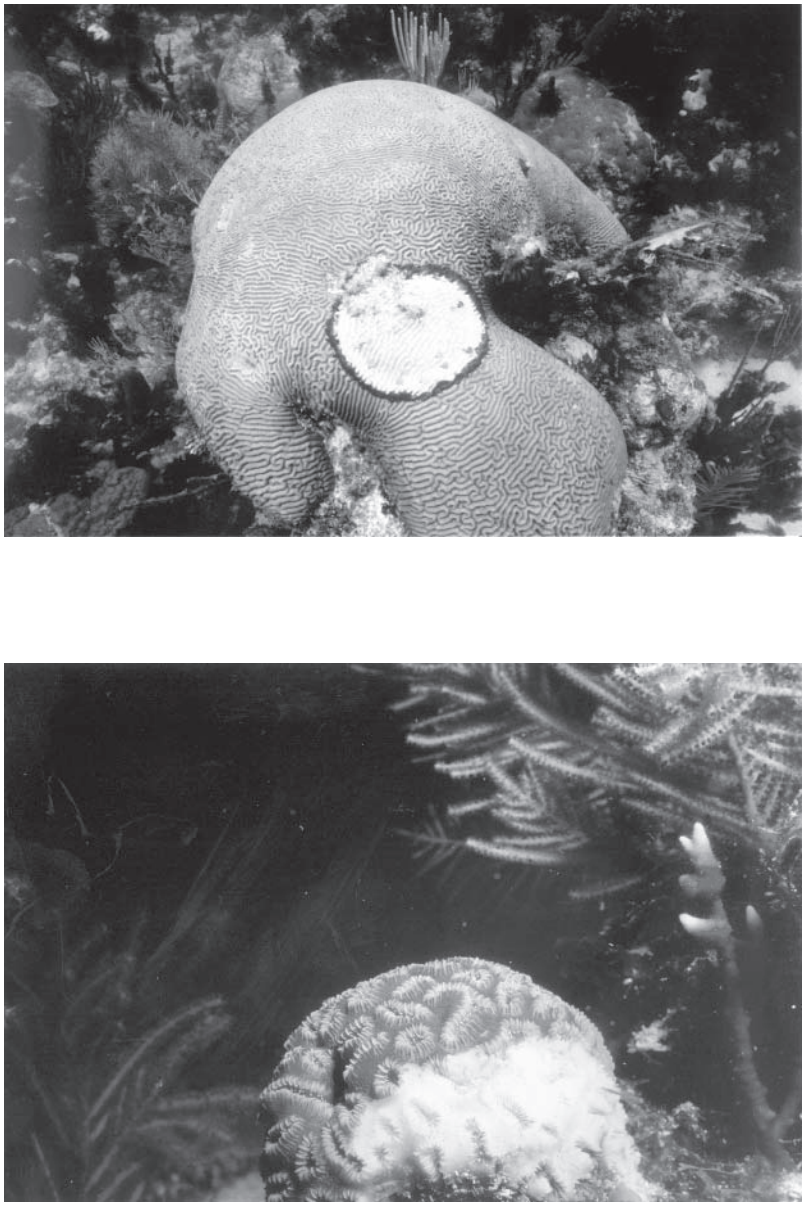

FIGURE 1 Coral diseases (top) black band and (bottom) plague type II contributing to the decline of coral reefs in U.S. waters. Courtesy of L. Richardson.

A different hazard to human health is exemplified by toxic dinoflagellates that seem to cause cognitive disturbances from exposure to aerosols of water that contained the organism (Grattan et al., 2001). Although understanding the chemical nature and pharmacology of this toxin may provide a useful experimental tool for neuroscientists, a more pressing need is to protect human health by developing assays for the toxin.

All of these areas are characterized by a real potential for rapid increases in understanding if modern molecular tools of biotechnology can be effectively integrated into more classical environmental disciplines. This integration will require interdisciplinary science and substantial funding, but the rewards should amply repay the investment.

Recommendations to Enhance Environmental Applications of Marine Biotechnology

- Explore the possibility that molecular tools will aid in understanding the environmental impact of oil and other contaminant spills in the marine environment. Molecular tools may also be used to track the progress of remediation efforts and may provide guidance for deciding when a remediation effort has ceased to provide clear environmental benefits.
- Bring genomic and other modern molecular techniques to bear on the nature and progress of diseases of marine organisms. Investments must be made to understand and develop methods for culturing marine pathogens. Similar tools will be essential for understanding coral-reef diversity as restoration proceeds.
- Develop genomic and other modern molecular techniques to monitor potentially toxic species, such as dinoflagellates, and human pathogens in the marine environment, so that potential outbreaks of disease can be predicted and eventually prevented.

In all these areas, it is essential that field and laboratory investigations go hand in hand and that the best of classical and modern molecular techniques be combined. That will happen only if interdisciplinary science is fostered and encouraged.

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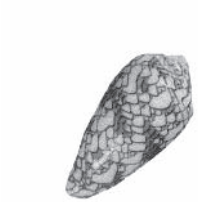
2001 Workshop Abstracts: Biomedical Applications



Keynote Address

FULFILLING THE PROMISE OF MARINE BIOTECHNOLOGY

Dr. Rita Colwell
Director, National Science Foundation

Marine biotechnology is a field with great potential, but with a distance yet to run. Over the past 30 years, we have seen incredible growth in many facets of genetics. Genetic information is used to produce new pharmaceutical products, disease-resistant plants, and modified microorganisms for industrial and environmental use.

Marine microbiology, however, in spite of some impressive discoveries, has essentially been left behind. These discoveries include hundreds of marine microorganisms and invertebrates with the potential of producing new pharmaceuticals, cosmetics, or nutraceuticals; new ways to raise fish, molluscs, crustaceans, and algae in aquaculture; and new ways to sense marine environmental changes. Yet, marine biotechnology is still a promise to be fulfilled while other areas of biotechnology have flourished both in science and in the marketplace.

The biotechnology pie can be divided into four major market segments: biomedical, agricultural, industrial, and environmental. Biomedical biotechnology is the most visible and has been since the introduction of a monoclonal antibody-based diagnostic test kit in 1981. This kit was the

first biomedical, biotechnological commercial product approved by the U.S. Food and Drug Administration (FDA). The following year, Genentech's recombinant human insulin (Rhinsulin; Humulin) was approved in the United States and is now used daily by more than 4 million people with diabetes around the world. From 1982 to 1989, only 18 biotechnology-based drugs were approved by the FDA; then the numbers rapidly increased with 22 new biotechnology drugs accepted by FDA in 1998 and in 1999. Thirty-one new drugs and vaccines were approved in the year 2000. The human population is reaping the benefits from this biotechnology explosion; there are now new drugs to treat herpes, rheumatoid arthritis, rattlesnake bites, diabetes, and cancers.

The agricultural sector has also experienced tremendous progress since the market introduction of the genetically engineered Flavr Savr tomato in 1994. Canola, corn, cotton, peanuts, potatoes, soybeans, sunflowers, and tomatoes are all more productive due to biotechnology. Many of these innovations are targeted at producing plants that are more resistant to insects and fungal diseases.

Though not yet as prolific as biomedical or agricultural biotechnology, industrial and environmental biotechnologies aim to minimize pollution and enhance materials and energy use, while maximizing production of recyclable or biodegradable products. To do this, microorganisms are bioengineered to degrade hazardous wastes, including chlorinated solvents, detergents, creosote, pentachlorophenol, and PCBs. Plants are induced to remediate organic and inorganic pollutants, including radionuclides. Enzymes that can function at extreme pH or temperature are being isolated and employed.

Through recent advances in genomic mapping, it is now known that humans, apes, and fruit flies are all closely related. Current science suggests that a minor difference in gene expression can make a major difference in structure, function, or longevity. As biotechnology adjusts gene expression to develop new products, this must be achieved with an eye toward scientific responsibility and good stewardship for the earth and humanity. When researchers leap into the unknown, they must use science as both a propellant and a safety net to predict where discoveries may lead and prevent adverse outcomes.

Marine biotechnology encompasses pharmaceutical, agricultural, industrial, and environmental applications. Although marine biotechnology is poised on the edge of a period of tremendous potential—potential for

discovery, potential for development, potential for design—the field is still in the realm of the future.

In 1985, I wrote, "There are several reasons for the lack of development in the area of marine pharmaceuticals. . . ." and then cited the difficulties of retrieving a sustained, reliable harvest of marine organisms; insufficient quantities of material to allow for study completion; and difficulties culturing marine organisms in the lab. Unfortunately, the same holds true today.

In the 1970s, recombinant DNA techniques were mastered, and unique microorganisms living in ocean-floor hydrothermal vents were discovered. It became clear that the application of genetic engineering to all forms of marine life formed a synthesis. The field of marine biotechnology was on the map and included production of commercially and medically important chemicals from algae and marine invertebrates; production of transgenic fish, crustaceans, and molluscs for food; and genetically engineered medicines and vaccines.

Seaweeds are an abundant source of food and food products, including carrageenan, vitamins, nutrients, and animal-feed additives. Chitin and chitosan, the polysaccharides derived from the exoskeletons of marine crustaceans, are used as gelling agents to control ice formation in frozen foods, as antifungal agents for agriculture, and as sutures and poultices in medical applications.

The discovery of many toxic molecules in ocean creatures indicated that the ocean was a likely source of pharmaceuticals. Despite nearly 40 years of research, there are only a few approved pharmaceuticals derived from marine organisms. These pharmaceuticals include materials originally isolated from marine sponges (the antiviral acyclovir, AZT, and the anticancer drug Ara-C) and cephalosporins, the antibiotics originally isolated from a pseudomarine fungus. Fifteen other compounds isolated from marine organisms, many of which were discovered with the assistance of the National Cancer Institute's Natural Products Branch, are in clinical trials or earlier stages of drug development. One anti-inflammatory substance, a partially purified pseudopterosin extracted from the Caribbean sea whip, the soft coral *Pseudopterogorgonia elisabethae*, has been licensed for use in skin-care products.

Hydroxyapatite, from marine coral, has FDA approval to be implanted into fractures or voids of human bones to aid in regrowth and repair. Horseshoe crab blood provides the basis of the limulus amebocyte lysate (LAL) test, which can test in less than an hour for endotoxin contamination in

medicines and medical appliances. Society needs many more of these quick-acting substances on which to base tests for human toxins and pathogens.

As I forecasted in 1983, the merger of genetic engineering with marine science created an opportunity for ocean research to provide products to improve humanity. Despite the advances in the identification and screening of organisms for biologically active compounds, production of sufficient amounts of the compounds depends on a number of factors. One is the ability to chemically synthesize the compound. Another is the ability to raise the organism in culture. A third is the ability to harvest the organism from its natural environment. All three of these issues can be seen in relation to the marine invertebrate *Bugula neritina.*

For example, it has long been hypothesized that *B. neritina,* a brown bryozoan animal, is not the true source of the antitumor compound bryostatin. Recent data from Scripps Oceanographic Institute indicated that the bacterium *Candidatus endobugula sertula,* which lives inside *B. neritina*, may be the agent producing this drug. If the gene is isolated from the bacterium, then biotechnology may provide a means for large-scale production of bryostatin for cancer treatment. Currently, modest production of bryostatin is achieved by limited mariculture.

Integrated mariculture systems allow land-based production of valuable bivalves, including *Mercenaria mercenaria*, the hard clam, a seafood of choice for many of us. Providing the proper environmental conditions—pH, temperature, oxygen, lack of toxins, or pollutants—while removing waste products like ammonia and organic and inorganic carbon, is a challenge that engineers are solving for improved productivity in aquaculture facilities. The challenges in developing mariculture are not solely for biologists.

Genetic engineering methods allowed cloning of genes from coelenterates to create products for cell biology research. Green fluorescent protein is a useful marker for tracking calcium in cells. The process has been "humanized" and cloned into mammalian expression vector systems.

Researchers in the Extreme 2001 Expedition, a deep-sea investigation, announced in Fall 2001 that they succeeded in conducting the first-ever DNA sequencing experiments at sea. Genomes of the inhabitants of superhot, hydrothermal vents almost 2 miles deep in the Pacific Ocean were sequenced. These organisms may yield new products ranging from pharmaceuticals to heat-stable, pressure-resistant enzymes for food processing or hazardous-waste cleanup.

To utilize the resources at hand best for achieving results that marine biotechnology promises, government must increase its involvement in marine biotechnological research. In the United States, for example, marine biotechnological research is funded mainly through the National Science Foundation (NSF), the National Oceanic and Atmospheric Administration (NOAA), and the Office of Naval Research (ONR). Smaller amounts of funding are also provided by the National Institutes of Health through the National Cancer Institute (NCI).

NSF's 1999 marine biotechnology funding reached $12 million. NOAA's portion was $10 million (about $8 million of which is earmarked for the National Sea Grant College Program), and ONR awarded $5.6 million for marine-related research. For the 2002-2007 budgets, NSF, NOAA, and ONR each will request annual increases of $10 million for marine biotechnology. They also will request an additional $3 million per year for outreach and education. The NSF, ONR, NCI, NOAA, and U.S. Department of the Interior, under the aegis of the National Science and Technology Interagency Biotechnology Research Working Group, Marine Biotechnology Task Force, have proposed a $50 million interagency initiative. This initiative, called COMPASS (Coordinated Marine Programs to Assess and Sustain the Sea), will be an interagency program to advance marine biotechnology, coordinate federal research and outreach in marine biotechnology, and address gaps in federal marine biotechnology funding.

At the NSF's Biological Oceanography Program workshop "Ecological Genomics: The Application of Genomic Sciences to Understanding the Structure and Function of Marine Ecosystems," it was suggested that a Virtual Marine Genome Center be established to contract out high-throughput genomics and to aid in the selection of organisms for sequencing. One of the requirements for the growth of marine biotechnological research is the increased use of genomics to learn more about the oceanic environment. More studies of ecology, symbiosis, and marine pathogens (such as my research group's continuing work on *Vibrio cholerae),* and production of biosensors must be performed. As more is learned, this scientific knowledge must be transferred to inform and educate the public, who are the people that fund and support our work. The public must understand biotechnology and not fear it.

Eighteen years ago, I thought that the marine biotechnology revolution was just about in reach. From the amount of research done in the field since then and the numbers of discoveries, I would say that, indeed, it was.

Nevertheless, from the small number of new products that exist today, I would have to say, the results of the revolution are unrealized. That is our challenge!

Today we are so much closer to the goal of realizing the sea's true potential, but we need coordinated national and international efforts and infusions of funds. Unlike 18 years ago, the technology has matured. I urge the participants in this workshop to put our combined knowledge to use and help society move forward to solve health and food-supply problems with the tools and research results of marine biotechnology.

Session 1: Drug Discovery and Development

ACCESSING MARINE BIODIVERSITY FOR DRUG DISCOVERY

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Nature has been the traditional source for organic chemical compounds used in medicine. For over 3,000 years, early societies recognized that their immediate environments were a rich source of plants that provided methods to treat ordinary infections, inflammation, arthritis, cancers, and many human diseases. Over the centuries, it became apparent that discrete chemical components of plants were responsible for these effects. It was not, however, until the seventeenth century that science would be sufficiently developed to begin to isolate, purify, and define these drug substances. Among the first pure drugs isolated were the powerful painkiller morphine purified from "tincture of opium," and aspirin, from the bark of the willow tree. Still today, especially in Asia, traditional medicines are prescribed and dispensed in ways similar to the historical past. However, many industrialized societies have moved in the direction of prescribing pure drugs with well-defined physiological effects.

The foundation of this "natural pharmacy" was the significant diversity of plant and, to some extent, animal life found in warm climates. Di-

versity was the key to a large number of chemically rich sources from which treatments were derived. Biodiversity translates to genetic uniqueness and diversity, which in turn relates to new biosynthetic pathways and potential. Although many thousands of plant species have been comprehensively examined using modern analytical methods, there still exist many thousands of terrestrial life forms that await investigation. That this endeavor is not antiquated is borne in the discovery of Taxol, the potent anticancer drug discovered in the bark of the Pacific yew tree. Given recent successes, there is every reason to expect that undiscovered drugs exist in the same plants and animals recognized to contain medications for over 3,000 years.

Although the diversity of life on land is great, the world's oceans are the center of global biodiversity, with 34 of the 36 phyla of life represented. The land, by comparison, is represented by only 17 phyla. Given this reality, drug discovery should have begun in the rich ecosystem of the oceans. Much of this diversity is found in the macroscopic plants and animals that are adapted to all the regions of the world's oceans (polar, temperate, and tropical). Species diversity reaches very high densities on coral reefs, occasionally reaching densities of approximately 1,000 species per square meter, particularly in the Indo-Pacific Ocean where tropical marine biodiversity reaches its peak.

Given the enormous biodiversity of the world's oceans, it is unfortunate that marine environments are the last great frontier for investigation. Unfortunately, with the pressures of economics weighing heavily on the pharmaceutical industry, natural product-based drug discovery has been characterized as encumbered and overly time consuming. Time will tell if alternative methods of accessing chemical diversity can replace this tried and true method.

Because the ocean is a much more demanding environment to sample, it is understandable that this ecosystem should be our last great biodiversity frontier. Over the past 30 years, marine plants and animals have been the focus of a worldwide effort to define the "chemistry" of the marine environment. Beginning in the mid-1980s, these efforts turned toward potential biomedical applications of novel chemical substances found in sponges and related colonial marine invertebrates. In this process, over 2,500 structurally diverse compounds have been found in marine plants and animals, and several of these compounds have been successfully interfaced with the pharmaceutical industry. Although no marine drugs have been developed as yet, several are in clinical and preclinical trials. Examples are bryostatin-1, ecteinascidin 743, dolastatin-10, and spongistatin for the treatment of

cancer. These compounds are novel both chemically and pharmacologically, and they hold considerable future promise.

The major biodiversity in the oceans, however, does not reside in the plants and animals, but in the enormous diversity of microbial life that can be found in marine waters, on the surfaces of plants and animals, and in the deep-sea sediments, which compose the major surface area of the planet. One milliliter of ordinary seawater contains 1 million microorganisms that are mostly uncultured and unknown. The surfaces and internal spaces of plants and animals are habitats that have been colonized by microorganisms as part of complex adaptations for survival. The bottom sediments, which are the repository of all organic matter in the ocean, are inhabited by a diversity of microorganisms, the complexity of which is only now being appreciated. Bacteria and fungi form the major classes found, but there are numerous other groups, such as the Stramenopila, which are essentially undefined. New actinomycetes are now being found as major inhabitants of marine sediments. Because these organisms reach densities of up to 10,000 cells per cubic centimeter, they might be the most abundant microorganisms available for drug discovery. Given the successful history of terrestrial microorganisms in the development of new drugs (over 120 marketed today), a systematic investigation of marine microbes is fully warranted. To achieve success in this endeavor, obstacles to the discovery and culture of these organisms must first be overcome. We must re-evaluate the marine environment, contrast it to the nutrient pools in terrestrial environmental and mammalian systems, and design new ways to isolate and culture marine microbes. It has become clear that marine systems harbor new genera and perhaps new major taxa of microbial life. We must meet the challenge to find ways to isolate and cultivate these organisms and thus to realize their contributions to the treatment of human disease.

MARINE NATURAL PRODUCTS AS A RESOURCE FOR DRUG DISCOVERY: OPPORTUNITIES AND CHALLENGES

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It has been stated that the "search and discovery of exploitable biology" is undergoing a "paradigm shift" as a "consequence of the bioinformatics

revolution" (Bull et al., 2000). In the context of natural product-based drug discovery, bioinformatics is producing new information that affects many of the key steps in the drug discovery process. Among the most significant developments are the revelation of vast new potential resources available from uncultured microorganisms and the discovery of a plethora of new potential therapeutic targets from various whole-genome sequences. This new knowledge presents tremendous opportunities for the discovery of therapeutic agents from natural sources. There remain, however, significant obstacles impeding the realization of this potential. Unlocking the biosynthetic capabilities of the new realm of marine microbes remains a fundamental scientific challenge. These organisms not only hold the greatest promise for the discovery of new agents from the marine environment but also provide a feasible solution to the inherent problem of supply. Studies of their physiology and means for their cultivation are vital. A major obstacle to the discovery of new marine natural products with promising biological activities is simply the difficulty of having them evaluated in a wide range of targeted assays. Although new therapeutic targets are being developed at an astonishing rate, ability to evaluate marine chemodiversity in these assays is severely limited. Part of the limitation owes to the scarcity of the compounds, which are often isolated in minute quantities insufficient to supply a library for repeated rounds of bioassay. Lacking a renewable source or reasonable synthetic route, many of these compounds will never have more than a few targets. The traditional process of natural-product discovery may preclude their evaluation against the widest range of biological targets, especially in ultrahigh-throughput-screening systems.

Through its component operations of Ayerst and the former Medical Research Division of American Cyanamid or Lederle Labs, Wyeth-Ayerst Research, the pharmaceutical research and development arm of American Home Products, has a rich history in the discovery and development of therapeutic agents from natural sources. At Lederle, the tetracycline family of antibiotics was the first product line derived from nature. Aureomycin (chlortetracycline) was isolated in the early 1940s from the soil organism *Streptomyces aureofaciens* and was followed shortly thereafter by four improved versions; the final one, Minocin, was introduced in 1971. Research has continued on this important class of antibiotics at Wyeth and a number of agents are advancing toward the marketplace. Two more recent microbial-derived commercial products are Rapamune (rapamycin) for use in transplantation and Mylotarg, a calicheamicin immunoconjugate targeting acute myeloid leukemia.

Wyeth's marine natural-products program has emphasized microbial sources since 1990. Collections have largely consisted of traditional isolation of microbes associated with marine habitats and organisms from tropical and temperate zones. One example, illustrative of both the challenges and opportunities, is the case of microorganisms isolated from the tropical marine ascidian *Polysyncraton lithostrotum. P. lithostrotum* was reported to produce the enediyne antibiotic namenamicin (McDonald et al., 1996), a compound having the same reactive chromophore as calicheamicin (Figure 2). With the initial goal of isolating the presumed microbial producer of namenamicin, a variety of microbes were isolated from the organism. In one set of experiments directed toward isolation of micromonospora, three such species were isolated, along with two fungi, three bacilli, one mycobacterium, two oceanospirillia, two pseudomonas, one rhodococcus and 10 others representing six genera. These organisms were cultured in liquid media and assayed for antimicrobial and DNA-damaging activities. A wide

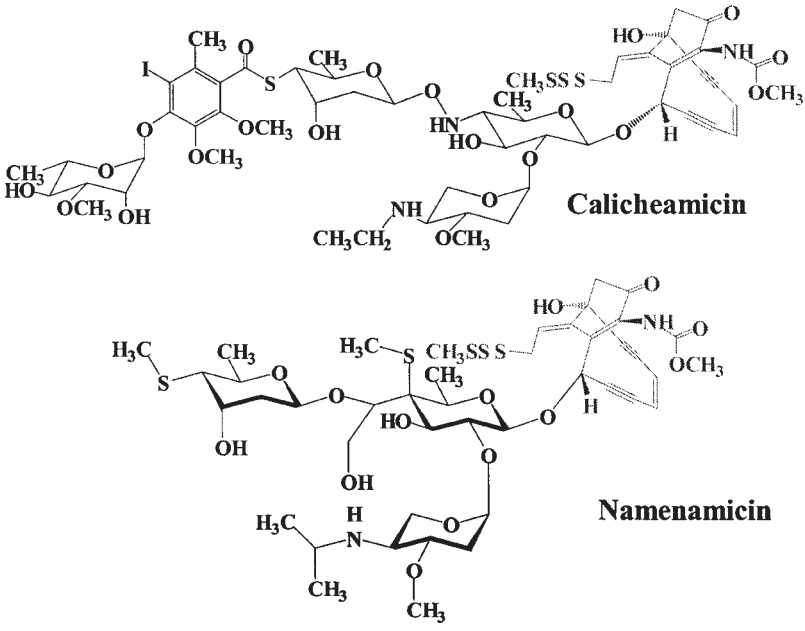

FIGURE 2 Microorganisms were isolated from the tropical marine ascidian *Polysyncraton lithostrotum,* which reportedly produces the enediyne antibiotic namenamicin (McDonald et al., 1996), a compound having the same reactive chromophore as calicheamicin.

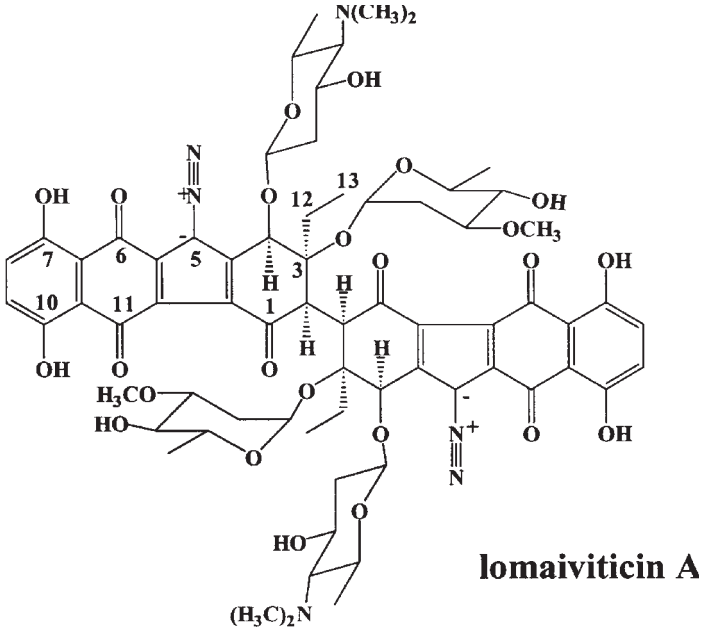

FIGURE 3 Lomaiviticin A was isolated from a previously unknown microorganism associated with a marine ascidian. The microorganism, tentatively named *M. lomaivitiensis,* appears to have potent DNA-damaging capability, thought to be caused by this compound.

spectrum of positive assay results led to the most promising candidate, characterized as a halophilic micromonospora species that produced potent DNA-damaging activity. Pilot plant scale fermentation of the organism, tentatively named *M. lomaivitiensis*, followed by bioassay-guided fractionation, led to the isolation of a unique dimeric bis-diazo compound lomaiviticin A (Figure 3) (He et al., 2001). Although lomaiviticin A has potent DNA damaging activity, it was not in the sought-after enediyne family. In fact, no namenamicin was detected from any of the isolates produced in these experiments. Although the initial goal was not achieved, a wealth of new organisms was obtained for further characterization as potential sources for new metabolites.

In addition to the realm of underexplored marine microbes as sources of new chemical diversity, a wide range of new opportunities exists for

natural product-based drug discovery. As new therapeutic targets continue to be uncovered through applications of bioinformatics, natural products can provide the greatest coverage of "chemical structure space" for evaluation against these targets. The challenge is how to realize this potential. With the shrinking timelines demanded by modern pharmaceutical research and development programs, natural product-based drug discovery faces additional hurdles that are not common to screening of synthetic compound libraries. These issues are not new, although their impact has become more substantial as the pace of screening and lead optimization processes has accelerated. Libraries of crude extracts were first created to allow natural products into the high-throughput-screening arena. Subsequently, purified compound libraries or peak libraries were introduced as a means of shortening the cycle time, and these are valuable improvements to the traditional process. Prepurification of extracts is one means for enhancing efficiency, but it also involves a considerable commitment of resources prior to initiation of screening. New technologies that can be used to quickly link target activity with a chemical species, i.e., bioactivity correlation, is an underdeveloped area. Robust and versatile affinity-based methods, e.g., those using chromatographic phases or size exclusion separation of target-bound ligands (Siegel et. al, 1998) would significantly advance reduced cycle times as well.

Many of the real or perceived bottlenecks to marine natural products-based drug discovery are founded on the issue of whether sufficient material will be available for complete biological and chemical evaluation and eventual production. Obviously, the development of synthetic and biosynthetic methods for production are real challenges, which must be addressed.

Studies directed toward understanding the biological roles of marine natural products should be encouraged. Too often, compounds with fascinating molecular structures are discovered and put aside without sufficient attention to their biological functions or mechanisms of action. Furthermore, if the mechanism of action correlates with a potential therapeutic role, then attempts should be made to define the critical pharmacophore via synthesis, chemical degradation, and/or modification. The research done on the hemiasterlin antitumor agents is an excellent example of studies of this nature (Anderson et al., 1997).

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MINING THE OCEAN'S PHARMACOLOGICAL RICHES: A LESSON FROM TAXOL AND THE VINCA ALKALOIDS

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Over the past 25 years the oceans have yielded a number of natural compounds that have led to the development of new and potent drugs for the treatment of human disease. These include the antiinflammatory drug manoalide, the cosmeceutical antiirritant pseudopterosin, and a number of drugs that are currently in clinical trials including the neurogenic antiinflammatory drug topsentin and the anticancer drugs bryostatin and ecteinascidin 743 (Faulkner, 2000; R. S. Jacobs, personal communication). Many other novel compounds have been isolated and characterized chemically, and preliminary biological testing indicates that they are interesting lead compounds for the future development of drugs for a wide variety of human diseases. Many of these compounds have structures that were not previously recognized by chemists as having pharmacological potential. A

cursory perusal of the recent literature provides a small sampling of the wide variety of compounds that have not been developed but have significant indications of efficacy (Table 4). Estimates of the number of existing compounds that are undeveloped is in the low hundreds (J. Faulkner, personal communication). One of the major stumbling blocks to the development of many of these compounds is that there is nothing currently known that allows us to distinguish them from the plethora of nonspecific toxins produced by a wide variety of organisms. This situation results from a lack of knowledge of their pharmacological mechanisms of action. A second major reason for their lack of development is their limited supply (Faulkner, 2000). Undertaking preclinical characterization and clinical testing of novel compounds requires hundreds of grams of compound, depending on its potency. Most of these compounds come from marine invertebrates or algae that are in relatively short supply. Thus, the choice is between culturing the organism in large quantities, developing a genetically manipulated culture system to produce the compound by means of modern molecular biological techniques, or chemically synthesizing the compound. Each of these production methods is costly and time-consuming. Sufficient scientific validation and information about the mechanism of the compound must be discovered first to develop significant advocacy and sufficient industrial interest for development.

One of the best examples of how this advocacy and interest-raising process occurs is the recent development of a number of antimitotic anticancer drugs, including Taxol and several vinca alkaloid-like drugs. Taxol and the vinca alkaloids are widely used and effective drugs that work by actions on microtubules. Microtubules are long proteinaceous tubules that form a dynamic and ever-changing skeleton or structural framework in the cell. They are central to many cellular functions, including cell movement, cell growth and reproduction, and cell signaling. It has been argued that microtubules are among the most important and most successful targets for anticancer drugs (Giannakakou et al., 2000). Although Taxol and vinca alkaloids are both derived from terrestrial plants, their development is a prime example of how the advocacy and developmental process works.

The history of Taxol in modern medicine starts over 30 years ago with the collection of samples of the Pacific yew tree by the U.S. Department of Agriculture (USDA) and the National Cancer Institute (NCI). Taxol's path from that point to its current status as one of the most successful new cancer drugs is the result of the perseverance of a cadre of chemists, pharmacologists, and oncologists (Horwitz, 1994), including seminal work on

TABLE 4 A Sampling of Undeveloped Marine Compounds with Significant Indications of Efficacy

Marine Source	Compounds	Potential Uses	Reference
Sponge *Trachycladus*	Onnamide F atode worm	Antifungal, antinem	Vuong et al., 2001
Sponge *Aka*	Kynureninase inhibitors serotonin sulfate	Neuroprotectants for use in AIDS-dementia and stroke	Feng et al., 2000
Cyanobacterium *Lyngbya*	Hermitamides A, B	Anticancer	Tan et al., 2000
Sponge	Axisonitrile-3	AntiTuberculosis	König et al., 2000
Sea whip *Pseudopterogorgia*	Pseudopteroxazole	AntiTuberculosis	Rodriguez et al., 1999
Sponge *Ircinia*	Cheilanthane sesterterpenoids	Kinase inhibitors, multiple uses	Buchanan et al., 2001
Fungus *Acremonium*	Oxepinamide	Antiinflammatory	Belofsky et al., 2000
Fungus *Acremonium*	Fumiquinazoline	Antifungal	Belofsky et al., 2000
Natural source	Polycyclic acridines	Drug resistant lung cancer	Stanslas et al., 2000
Hydroid	Tridentatol A	Antioxidant inhibits LDL lipid peroxidation (superior to vitamin E)	Johnson et al., 1999
Ascidian	Lamellarin alpha 20-sulfate	AntiHIV virus	Reddy et al., 1999
Microorganisms	Cyclic depsipeptide Sansalvemide A	AntiPox virus (MCV)	Hwang et al., 1999
Natural source	Makaluvamines	Anticancer	Matsumoto et al., 1999
Crinoid	Gymnochrome D	Antidengue virus	Laille et al., 1998
Fungus *Phoma*	Phomactins	Antagonist of platelet activating factor	Sugano et al., 1996
Sponge *Cymbastela*	Diterpenes and others	Antimalarial	Wright et al., 1996
Xetospongia, *Agelas*	Xestospongine B, sceptrine, age	Cystic fibrosis, impotence, Alzheimer's, cancer	Vassas et al., 1996

its mechanism by Horwitz and the support of the NCI. Although interest in novel microtubule-active drugs was at a low point 20 years ago, Horwitz and her collaborators discovered that Taxol had the unusual characteristic of bundling microtubules at high drug concentrations rather than destroying them as the vinca alkaloids were known to do. The novelty of this observation provided the needed push to encourage development of a drug that has become one of the success stories of modern pharmacology. Subsequently, there has been a rush to develop improved taxane-like molecules, which has led to a strong industrial interest in a number of marine natural products, including eleutherobins, sarcodictyins, and discodermolide (Faulkner, 2000; Jordan, 2001).

The story does not end there. In more recent developments, we have been studying the effects of Taxol and vinca alkaloids on the very important dynamics of cellular microtubules. We have found that although there are important differences between the actions of Taxol and the vinca alkaloids involving their effects on microtubule mass at high drug concentrations, at another mechanistic level, surprisingly, they act similarly to suppress microtubule dynamics (Wilson and Jordan, 1995; Jordan and Wilson, 1998; Jordan, 2001). Both classes of drugs, the microtubule stabilizers and the microtubule depolymerizers, act at very low but physiologically relevant concentrations to stabilize the dynamics of microtubules in dividing cells. We have found that the stabilization of microtubule dynamics blocks cancer cells in mitosis at a well-defined stage of the cell cycle and sends the cells into a death program known as apoptosis, thereby killing the cancer cells. These recent discoveries have led to the industrial pursuit of a number of similar compounds from the sea, including the dolastatins and halichondrins. These drugs are currently in clinical trials for cancer or are scheduled for clinical trials. Other microtubule-active agents, such as curacin A, have high potential but have encountered stumbling blocks that can be overcome with further research.

Despite their success and efficacy, the current microtubule-active drugs have significant shortcomings. They are useful in treating only specific kinds of cancer, and patients often become resistant to these drugs, the result being that the cancer eventually returns with a vengeance. We desperately need novel cancer drugs that will be effective against a number of very resistant tumors, such as kidney, pancreatic, and brain tumors. The large number of undeveloped marine compounds holds promise for filling this need.

Each of the antimicrotubule drugs discovered so far acts differently on

tumors and many of them bind to unique sites on microtubules. Several of the newer drugs overcome drug resistance that is a major limiting factor in current chemotherapy. We are optimistic that the oceans will yield undiscovered drugs that are specific for currently untreatable forms of cancer. This can only occur with additional funding in the area of marine pharmacology. (Supported by National Institutes of Health National Cancer Institute #57291.)

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ECOLOGICAL ROLES: MECHANISMS FOR DISCOVERY OF NOVEL TARGETS, COMPARATIVE BIOCHEMISTRY

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For nearly a century, the field of Comparative Biochemistry and Physiology has been driven by one unifying theme, namely the "August Krogh

principle." To paraphrase this early-twentieth-century Danish physician and physiologist: for every experimental problem, there is an organism which is ideally suited for its experimental study. A familiar example is the elucidation of the fundamentals of the action potential of nerve cells through use of the squid giant axon since the 1950s. This presentation examines how the principles of studying species diversity that are central to comparative biochemistry and physiology might be further applied to the field of marine natural products discovery.

Many examples of the August Krogh principle now exist in comparative biology and medicine, and the generally accepted notion of the utility of marine and freshwater animal models of human disease states and processes was recently reviewed in the National Research Council's report *Monsoons to Microbes* (NRC, 1999). It is helpful to review some of the features of aquatic organisms that make them good experimental subjects to complement the direct use of mammalian systems:

1. *Fish and invertebrates represent a vast phylogenetic diversity, much of which is marine, that far exceeds that of mammals.* Because these many aquatic species have been waging "chemical warfare" with each other for millennia, their susceptibilities to natural environmental agents are often different from those of mammals, and there is likely to be considerable variation among aquatic species. These differences can be exploited to discover the underlying unifying mechanisms of toxicity and effect. In essence, unraveling of mechanisms in mammalian systems can be hastened by a comparative toxicological and pharmacological approach. Often the aquatic model is simpler and can give the scientist a "stripped-down" version of a more complicated mammalian system. Sometimes models are more sensitive to critical toxins than mammals, and sometimes they are less sensitive. If species choices are made carefully, a great deal of information about a natural product can be gathered simply by changing species as the experimental variable. I will return to this point below.

2. In applying a comparative approach, *aquatic species offer a simpler, natural, intensive exposure system,* because respiratory surfaces, skin, and fin surfaces (which lack keratinization) can be bathed directly in water with the substance of interest. Specifically in marine fish, their constant osmoregulatory demands to drink water ensure that gut throughput and exposure is high. In the context of development, embryos can be exposed directly.

3. Because fish and invertebrates naturally experience body-tempera-

ture changes, *the effects of temperature on chemical exposures can be directly and realistically studied in these species*.

4. Many marine organisms are extremely fecund (with eggs numbering in the tens of thousands or more), have external fertilization and short generation times, and are easily mass cultured, often at a lower cost than that of maintaining mammalian colonies. Therefore, these reproductive features provide *enhanced opportunities for genetic research and manipulations*, where developing embryos can often be directly observed. The use of zebrafish in vertebrate developmental studies is a strong example of this point.

5. Given the above experimental advantages and the heightening pressure to use fewer mammals (and animals in general) in biomedical research, aquatic organisms offer *opportunities to conduct at least some natural products-related research in a much more cost-effective and socially acceptable manner*.

Returning to the first point, I'd like to propose that for several reasons the species diversity in the marine environment itself may be a desirable source of experimental variation for the organisms upon which natural products are tested. First, millennia of chemical warfare have produced organisms that are *immune* (or at least less susceptible) to a particular natural toxicant. Has the organism achieved this immunity by modifying the target molecule (e.g., receptor proteins and ion channels) to avoid the effect of a natural toxicant? Alternatively, is the organism especially good at metabolizing or excreting the natural toxicant? Note that both of these or other responses could be true because the species expresses a novel gene product (e.g., metabolic enzyme or drug transporter), because it over- or underexpresses a relatively standard gene product, or because it has modified proteins of the target or elimination pathways with a simple post-translational modification. The adaptations learned from study of these natural experiments in natural product resistance might be excellent predictors of how resistance might be achieved by the ultimate target cells in mammals. These cells (e.g., bacteria, fungi, or even cancer cells) have much shorter generation times (and in the case of cancer cells, genetic instabilities) and are notorious for rapidly acquiring drug resistance. The information from natural adaptations might allow a researcher to design a synthetic analog of the compound in a very targeted way to make it less prone to evolution of alternative metabolism or excretion paths.

In the post-human-genome era, focus has now turned from sequenc-

ing "the" genome to the discovery of individual variation in DNA sequences (e.g., the so-called SNPs, or single nucleotide polymorphisms). The hope is that in addition to individual SNP profiles being predictors of disease onset, a physician might also be able to advise patients on the basis of the profiles that they are more or less susceptible to the beneficial effects or the side effects of a drug. A key question is how will these differential susceptibilities be determined? Certainly not by direct experimentation on humans, at least not in the early stages of drug testing. Given how complex the factors are that govern the accumulation of mutations within a species, it is not likely that the exact same mutations one sees in humans will always be readily available in common mammalian test organisms (e.g., mice and rats). This lack of key genetic similarity is often seen in mouse trials in which a drug that is promising never pans out in human trials. However, given the much larger genetic palette of aquatic organisms to choose from, the chances of finding a test organism with an identical or nearly identical mutation to a human SNP increases dramatically. Thus, one might use a species from the marine environment that is noted for being susceptible to the side effects of a drug to do some initial screens to see what human subpopulations might have problems.

Clearly, a key to using marine species in the ways suggested above and below will be the increased availability of genome sequences. Theoretically, the more genome sequences from novel organisms that are available, the more likely it will be that an analog to mutation X in gene Y can be found in a convenient test organism. Additionally, once a large panel of cDNA sequences (or ESTs, expressed sequence tags) from alternative species are available, functional genomic and toxicogenomic experiments with natural products are possible. Thus, one can expose nontraditional organisms to a test compound to see what genes are upregulated or downregulated. Likewise, as proteomic databases are developed for aquatic organisms, the responses of their proteomes to test compounds can be examined. One useful aspect of these sorts of comparative approaches is in finding the common actions of a test compound in all species. In drug discovery, a common complication is that a given compound can affect many pathways. By using functional genomic and proteomic approaches to see what genes and proteins are commonly affected across all species, the utility of the compound (or again, its specific derivatives) can be determined.

Genomic approaches can also aid in the discovery or synthesis of the natural products themselves. As the proteins of metabolic pathways for natural products are further elucidated, and the genes for these pathways

are characterized, it is quite possible that organism X can be predicted to synthesize compound Y, or something like it, or that *Escherichia coli* or other "standard" organisms can be engineered to make compound Y. Suppose that a particularly potent drug is found to be derived only from a rare species (or one that eventually becomes protected, endangered, or even extinct). If we can compare its genome, proteome, or functional genomic characteristics with those of other species, we might easily find the gene or genes responsible for making compound Y and then be able to find alternative source species or to make it enzymatically or *in vivo* in an engineered organism.

At first, the actual aquatic species used in all the above suggestions would be limited to those for which genomic and proteomic work are proceeding for other reasons. Fortunately, these approaches are growing in popularity and coming down significantly in costs, so the species list will grow rapidly. The types of species whose genomes and proteomes are being studied seem naturally to fall into the categories of "stress susceptible" (e.g., rainbow trout) or "stress resistant" (e.g., killifish, mudsuckers, and toadfish). The state of genome research for some representative aquatic species will be presented, and one topic of discussion might be what species should be further targeted for research that will make them useful to natural products discovery.

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THE INTERFACE OF NATURAL PRODUCT CHEMISTRY AND BIOLOGY

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Natural chemical constituents of living organisms called natural products have historically been used to treat human infections and diseases. As the discovery rate of new biologically active natural products slows in comparison to the increased rate of infectious diseases that are developing resis-

tance toward traditional antibiotics, it is imperative that the discovery rate of novel drug candidates increases. To ensure the constant flow of new chemical entities for drug discovery, techniques in molecular biology have merged with those in natural product chemistry to facilitate the generation of novel and rare natural products through combinatorial biosynthesis, metabolic engineering, and accessing the biosynthetic potential of uncultured microorganisms.

The field of combinatorial biosynthesis exploded after the realization that natural product-based chemical libraries can be created through biotechnology and that the field has the potential to dramatically alter the way natural product drug leads are investigated and developed (Hutchinson, 1998). The approach involves the expression of secondary metabolic biosynthetic genes from one or more systems in an alternative host to create unnatural metabolic pathways that result in the production of "unnatural" natural products (Cane et al., 1998). The success of the combinatorial approach to structural diversity relies upon drawing from different classes of biosynthetic pathways. In other words, combinatorial biology is largely limited by the breadth of available biosynthesis genes and the diversity of reactions their products catalyze. To date, the majority of effort has centered on engineering polyketide and non-ribosomally derived peptide-based metabolites (Cane, 1997). Tailoring reactions involving glycosylation, oxidation, methylation, and acetylation are now used to add further structural diversity to the engineered libraries.

As natural products from marine microorganisms are emerging as a new source of novel structures with little overlap from traditional sources (Fenical, 1993), they present an opportunity to develop a new generation of recombinant compounds by expanding the combinatorial biosynthetic repertoire to include novel biosynthesis genes from marine systems. To date, however, only two marine natural product biosynthetic gene clusters, the bacteriostatic polyketide enterocin (Piel et al., 2000) and the polyketide and peptide microcystin (Tillett et al., 2000), have been cloned and sequenced. Additional marine microbial natural product biosynthetic pathways are being sequenced in several laboratories and are certain to provide the genetic tools to extend this technology into new areas, including terpenoid and halogenation biochemistry. As a consequence, hybrid-engineered small molecules derived from mixed biosynthetic pathways are likely to have biological properties not addressed by polyketides and peptides alone, thus expanding combinatorial biology into new therapies.

Natural products from marine invertebrates additionally expand the

chemical diversity available for biotechnology, and promise to be another excellent source of novel genetic tools. In many cases, microbial symbionts hypothetically biosynthesize natural products isolated from marine invertebrates, particularly the sessile ones (Moore, 1999). Given that marine invertebrates can be rare, difficult to collect, and slow growing and that their removal from the environment might have negative consequences, marine biotechnology is well positioned to circumvent these problems through the cultivation of symbionts and the genetic engineering of biosynthetic machinery in heterologous hosts. Molecular phylogenetic analyses of the microflora of marine sponges from different oceans, for instance, have recently revealed uniform microbial communities distinct from marine plankton or sediments (Hentschel et al., *submitted*). A picture is emerging where sponges may be viewed as highly concentrated reservoirs of uncultured, elusive, and possibly evolutionarily ancient marine microorganisms that have not been utilized in drug discovery programs. Major challenges will involve the development of new methods to access the biosynthetic potential of these microorganisms through cultivation and heterologous expression of clustered secondary metabolic pathways.

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Session 2: Genomics and Proteomics

HIGH-THROUGHPUT CULTURING FOR MICROBIAL DISCOVERY

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It is estimated that less than 1 percent of the earth's microbial life can be grown using standard agar plating techniques; of over 40 known prokaryotic phyla, only 23 have cultured representatives. Uncultured microorganisms are a vast reservoir of biodiversity from which new small molecules and enzymes can be recruited for applications in medicine, industry, and agriculture. To culture novel microorganisms, we developed high-throughput-culturing (HTC) procedures to isolate cells in very low nutrient media. This approach was designed to address microbial metabolic processes that occur at natural substrate concentrations and cell densities, which are typically about three orders of magnitude less than those in common laboratory media. The approach makes use of microtiter dishes and a newly developed procedure in which cell arrays are made on microscope slides and screened by fluorescence in situ hybridization (FISH). Approximately 2,500 cultures of pelagic marine bacteria were examined over the course of 3 years and 14 separate samplings. Up to 14 percent of cells from coastal seawater were cultured using this method— a number that is 1,400 to 140-

fold higher than that obtained by traditional microbiological culturing techniques. Among the cultured organisms are many unique cell lineages that will be named as new species and genera by microbial systematists. Ninety percent of the cells recovered by the project in these early experiments do not replicate in Petri dishes of agar media, the most common method of microbial cell cultivation.

THE GENOMICS REVOLUTION: CHALLENGES AND OPPORTUNITIES

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The genomics revolution has provided the DNA sequence for nearly 60 microbial species and a number of important animal and plant species, including human. This information provides the foundation from which a new, comprehensive, and profound understanding of the biology of living systems, the history of life on earth, and the role of genes in disease and disease susceptibility can emerge. Genome-enabled studies on microbial species have revealed new insights about mechanisms of microbial evolution, novel metabolic capabilities, novel approaches to the diagnosis and treatment of infectious disease, and potential biological solutions to environmental remediation and alternative energy sources. Genome sequencing still remains the most robust method for assessing the overall gene complement of any organism, and as costs for DNA sequencing have dramatically decreased, the possibility of using this approach to study new species and microbial populations has become more realistic. It is important to keep in mind that all the work done to date in microbial genomics has focused on species that can be cultured in the laboratory or grown in animal cells. However, uncultured species, particularly from the marine environment, should be a priority for future genomic studies, and the technology now exists to allow us to think about microbial-community genomic projects. One of the most profound lessons that we have learned from the genomics revolution is how little we actually understand about the biology of life on earth. With these immense data sets in hand, we will now be able to pursue avenues of research that were impossible just a few years ago. Although the benefits of this new understanding are apparent, the path forward is formidable. This goal will require the marriage of pow-

erful new technologies from the fields of biology, mathematics, computational biology, engineering, and physics to achieve an understanding of systems biology. Moreover, it will require that investigators entering the life sciences as well as established investigators be provided opportunities to receive training in genomic analysis and bioinformatics to fully exploit the information that is being compiled in numerous databases around the world.

MICROBIAL GENOMICS: WHERE DO WE GO NOW?

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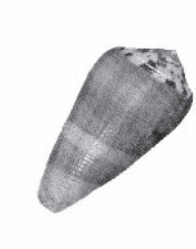
Since its beginning in 1994, the U.S. Department of Energy (DOE) Microbial Genome Program has sparked a revolution in microbiology. To date, complete genome sequences of approximately 52 microbes have been published; sequencing of at least a dozen more is known to be complete but not yet published, and sequencing projects of approximately 140 additional microbes are known to be in varying stages of progress. Activity in the private sector has also been intense. Sequencing technologies have progressed to the point where a high-throughput facility, such as the DOE Joint Genome Institute, can draft the sequence of a 2.5 Mb microbe in 1 day and about 65 Mb of sequence in 1 month, and this productivity is rising. This torrent of sequences is enabling a variety of new discoveries. These include new genes and pathways, and the insights that horizontal transfer of genetic information might have been frequent in microbial evolution. Genes of unknown function are astonishingly frequent. Microbes have been isolated (and their genomes sequenced) from extreme environments characterized by low pH, temperatures above boiling water, pressures greater than 200 atmospheres, highly toxic metal concentrations, high radiation fluxes, high salinity, and just about every other inhospitable condition imaginable. Most microbes do not cause diseases and, in fact, their important roles in maintaining the ecology of the earth are becoming clearer. Their sequences will contribute to a deeper and richer understanding of microbial life on the earth.

DOE is planning to take the next steps with its ambitious Genome to Life initiative. Its thrusts are four: (1) Understanding protein complexes

will increase the number of targets for pharmaceuticals, and will increase the numbers of points of intervention in cell functioning. (2) Better understanding of regulatory networks will also help in modifying cell function in ways that potentially can be useful in addressing mission needs. (3) Exploring functional diversity will add to the limited repertoire of microbial mechanisms for adapting to a variety of environments and for interfacing with the spectrum of substances, both organic and inorganic, that microbes encounter. After all, microbes have been on earth for more than 3.5 billion years and have learned to thrive in many niches exploiting available energy sources and nutrients. (4) The ability to model cell behavior *in silico* will generate many testable hypotheses (much as gene sequences do today) for a much deeper understanding of cell structure and function.

Although enormous value is still to be gained from continued sequencing, we now need to learn how to put the biological "parts" together into understandings of cell processes and functions. The familiar (and very successful) reductionist approach needs to be supplemented by a new "reconstructionist" approach that recognizes that complex biological systems are more than the simple sum of their parts. Starting with "simple" microbial cells and being aware that multicellular life evolved from unicellular forms, we can expect this to be a massive challenge for all of biology.



Session 3: Biomaterials and Bioengineering

THE COMMERCIALIZATION OF A BIOPOLYMER EXTRACTED FROM THE MARINE MUSSEL, *MYTILUS EDULIS*

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In turbulent intertidal zones throughout the world, such marine animals as barnacles and mussels tenaciously attach themselves to slippery wet rocks, pilings, or any solid surface. The adhesives they use are remarkably effective. Scientists have researched these natural adhesives with a vision toward the development of new products for applications in science, medicine, and industry. Marine biologist J. Herbert Waite discovered the active ingredient of the mussel adhesive. He named it "polyphenolic protein." This molecule forms strong, flexible bonds on virtually any surface, natural or manufactured, wet or dry. The secret of its versatility is the protein's unusual chemical composition. Not only does it readily interact with manufactured materials, such as metals and plastics, but it also bonds to living tissue, such as bone and skin. Because it is a protein, the adhesive is gradually broken down by natural processes within the human body. This property of resorbability, together with a setting time that can be adjusted

as needed for specific uses, suggests that the polyphenolic protein could be the key component in an effective surgical adhesive.

BioPolymers' strategy was to commercialize specially designed adhesive formulations through licensing, cooperative development programs, and joint ventures with companies in the targeted markets. Customers in biomedical research have been using the adhesive to simplify the manipulation of cells and tissue outside the body.

BioPolymers, Inc. spent 5 years focused on developing and commercializing novel adhesives and coatings for the medical and industrial markets. That concept was spawned in 1985 after Dr. Waite's discovery of a decapeptide (sequence of 10 amino acids) which repeated 85 times to comprise the mussel adhesive protein (MAP). After licensing this technology from the University of Connecticut (patents 4,585,585 and 4,687,740), the company selected the patented 10 amino-acid sequence and variations of it for commercial development, although it was not known at the time which amino acid in that sequence was the proper starting point for the decapeptide.

The mussel in its sea environment adheres to many substrates, and laboratory research indicated that extracted and synthetic protein polymers could stick to almost any substance, such as rock, plastic, metal, glass, Teflon, and skin. Even though the original concept was never demonstrated to work as an adhesive or coating in a medical or industrial environment, the fact that the mussel anchors itself by a collagen byssus thread to these surfaces supported the notion that the MAP could also adhere to the collagen of biological tissue. Proof of that theory was part of the responsibility of the new company.

During its first 2 years, BioPolymers extracted the natural MAP and developed a product, CELL-TAK, to enhance cell attachment and, where appropriate, cell growth, in laboratory culture dishes. However, the market was small, and financial return too limited to warrant further expansion in this area. Hence, a distribution agreement was signed with another cell-culture product company. Also, during the first 2 years, the company's attention focused on the medical field. A patent application was filed covering a myriad of end uses for formulation of mussel-based adhesives.

In 1986, BioPolymers also conducted gross feasibility tests of the MAP in several ophthalmological *in vitro* and *in vivo* models. Some were successful, but recognizing the limitations in the supply of mussels and the degree of difficulty in moving a natural material through the FDA's regulatory approval process, a significant research and development emphasis was

to focus on developing a synthetic version of MAP. The initial breakthrough was accomplished in late 1987 by attaching the chemically synthesized decapeptide to a backbone to form a copolymer with high molecular weight similar to natural MAP. By mid-1988, a cross-linking agent was found, and when combined with the synthetic MAP, appeared stronger than cross-linked natural MAP in *in vitro* studies on attaching skin to skin and cornea to cornea. By the fall of 1988, short-term experiments to develop specific formulations for wound management (the largest medical market opportunity) began *in vivo* in pigs. Results were very encouraging; animal shear strengths were more than sufficient to hold a skin graft in place over its entire surface area. When testing was expanded beyond the initial period, the experiments failed; strengths were at or below the level of controls. In drier, lower stress biological systems, such as the eye, the adhesive bond survived significantly longer for closing corneal perforations in rabbits and for corneal-type transplants in primates. Unfortunately, neither bond was sufficiently long enough to complete the healing process.

BioPolymers entered 1989 focused on reformulating the adhesive components and retesting the combinations with the assumptions that the original "ingredients" were correct, but the "mix" might have been wrong for the *in vivo* environment. Continued experimentation, scientific analysis, and consultation with several expert scientists in related fields failed to pinpoint the exact cause of failure. Before it could expect success with a synthetic version, the evidence and collective opinion suggested that BioPolymers had to complete its understanding of how the mussel attached to objects and, in particular, to identify other components that work with the natural MAP in the adhesion and coating process.

Primary Research Areas

Basic Mussel Research

Although the decapeptide was originally identified as the functional component of the adhesive, it is now believed that other components are also necessary. Working with Dr. Waite at the University of Delaware and researchers at the University of Connecticut Health Center, BioPolymers undertook the process of isolating and identifying the other components. Perhaps these "missing" components could better utilize DOPA, the amino acid in the protein, in *in vivo* animal studies.

- BioPolymers' synthetic and natural MAP formulations have exhib-

ited acceptable shear strength, but consultants in adhesive technology reported that an adhesive must also have peel strength. Although the mussel's adhesive in nature is observed to have peel strength, current formulations, as measured in the laboratory, do not. This may be further confirmation that components to bind with MAP are missing.

Conformation of the Decapeptide

- An outside effort at the University of Illinois found that the decapeptide on the backbone, as it was synthesized, may not be able to fold into the correct shape as natural MAP to attach to the collagen in skin or bone. More amino acids may be needed in the peptide—not 9 or 10 as it was designed.
- There were indications that the decapeptide should have started with a different amino acid in the chain to provide the proper shape to integrate with collagen.

Novel Polymer Synthesis and Formulation Development

- For the reasons cited above as well as other characteristics of the synthetic MAP, the limited successes achieved with the synthetic MAP did not effectively utilize DOPA—the proprietary technology—in the curing process.
- Formulation research should include wound bed stabilizers, as are found in other tissue adhesives, such as fibrin.

If these technical tissues were resolved, new components identified and utilized in formulations, the research and development effort could move quickly into testing, because the comprehensive *in vitro, in vivo*, and safety models developed are still applicable and relevant.

The safety and efficacy testing protocols were considered to be significant assets of BioPolymers. The water-based, proteinaceous polymer adhesive system being developed at BioPolymers required significant adaptation and test development to achieve reliability and reproducibility in results. A large investment in research time and consultation by adhesion test experts resulted in well-defined protocols for *in vitro* and *in vivo* tests. With further research, new formulations and modified polymer or cross-linking systems could readily be evaluated against a large body of data using the established test systems.

Industrial Applications

Technology Review Status

In 1990, a research and development project to investigate the industrial applicability of MAP was initiated in the areas of anticorrosion coatings and metal sequestering (for contaminated wastewater and metal reclamation). Early experiments focused on the development of protocols, model systems, and initial feasibility studies.

Specialty Coatings

To test the capacity of DOPA-containing polymeric systems to protect metals against corrosion, development was underway to establish techniques for cleaning surfaces of metals, such as copper, carbon-steel, and aluminum.

Because cost is the driving force in the protective coatings area, a search of the literature for inexpensive, readily available, water-soluble polymeric substances that could be converted to DOPA-containing coating systems was completed. Several polymers were identified and toll vendors were being sought. After these fundamental anticorrosion data were acquired, the plan was to use a number of specialty ink and coating formulator consultants.

Metal Sequestering

Because of the strategic nature of certain rare metals required in high-tech military manufacturing procedures, protocols for the sequestering of Group VI metals from solution were being developed. Some of the metals targeted for chelation included tungsten, molybdenum, chromium, and vanadium. Early experimental results were very promising. A 200-fold decrease in concentration from a solution of these metals was accomplished with supported heterophase DOPA and DOPA analog complexing agents.

Unfortunately, a cursory literature search of the catecholate metal complex patent revealed a large body of previous art. However, specific patent searches on supported catecholate complexing agents did not identify previous art that would preclude patent protection for this technology. Further, examination of supported polymeric sequestering agents that would fall beneath the umbrella of U.S. Patent 4,908,404 still needed to be con-

sidered. In addition, a great deal more experimental work was required to capitalize on the experimental advances already accomplished.

Biopolymer Patents Issued

Adhesives Derived from Bioadhesive Polyphenolic Proteins (No. 5015.677, 1991)

This invention relates to adhesive formulations that are derived from bioadhesive polyphenolic proteins and used in a wide variety of applications. This claim currently covers a formulation that has a bioadhesive polyphenolic protein component with a specific weight percent of a proteinaceous substance comprising about 50–150 repeating units of the decapeptide and from about 1 to 40 percent of a cross-linking agent that promotes cross-linking of the decapeptide. The formulation optionally also includes additives that promote desired properties of the formulation and fillers.

Synthetic Amino Acid and/or Peptide Containing Graft Copolymers (No. 4,908,404, 1990)

This invention relates to a peptide-containing graft copolymer with a molecular weight of about 30,000 to 500,000. It includes a polymer backbone containing or capable of modification to include free primary or secondary amine functional groups for reaction with an amino acid or peptide graft and an amino acid or peptide graft reacted with at least 5 percent to 100 percent of the primary or secondary amine groups on the polymeric backbone. The amino acid or peptide graft comprises at least one dihydroxyphenylalanine (DOPA amino acid) or a precursor thereof capable of hydroxylation to the DOPA form.

SELF-CLEANING SURFACES: BIOLUBRICANTS, DRAG REDUCTION

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Imagine yourself cruising in a sleek, new fishing boat off the Atlantic shore, making good speed, when suddenly porpoises, so common in these waters, begin to frolic near your boat. Although you are going as fast as you can, these friendly marine mammals probably will not only keep up with you, but will swim circles around you, even without riding your bow wave.

After some six months in the water, you will find that your vessel will not be able to make anywhere near the top speed it did when it was freshly launched with a new coat of antifouling paint. However, the porpoises and their cousins, the killer whales, who will have been in the water much longer than your boat, will have no trouble maintaining their speed. (Baier and Meyer, 1986).

The Marine Environment

Marine mammals, fish, and other organisms have evolved to display different techniques to reduce drag and increase "lubrication." Many of these same organisms also remain relatively free of biological fouling throughout their lives or during critical stages of their development. Certainly, the sleek shapes of many of the swimming creatures contribute greatly to their ability to move through the water easily, while expending relatively little energy. Textural features also may play a role in the reduction of "form drag." The circumferential ridges that dolphins can produce in their skin, for instance, may serve to increase the drag-reducing boundary layer around the quickly moving mammal.

Research and development of synthetic forms and materials primarily has mimicked the macroscopic forms and textures of marine mammals and fish. This approach has been well exercised for more than a thousand years for the design and use of fishing and exploration vessels and, more recently, submarines, torpedoes, and ocean-drilling platforms.

The study of the micro- and submicroscopic tools developed by nature for drag reduction and lubrication is a more recent phenomenon. At the microscopic and molecular level, characteristics of the water-contacting surfaces of low-drag and fouling-resistant marine organisms and plants fall

into two general categories: those that reduce drag and fouling by sacrificial slime, exudate films, or tethered macromolecules on their surfaces; and those that achieve these effects through the intrinsic low-surface-energy of their cellular and tissue surfaces. The development of fouling-release paints and coatings over the past 30 years has produced a marked reduction of drag along ships, primarily due to the significant decrease in hard fouling (e.g., barnacles and tubeworms) of these coatings. Is there, however, another significant increment of improvement that could be obtained from additional study of natural marine organisms and surfaces? And how can these findings be translated to the biomedical marketplace?

The Biomedical Environment

Although work on tethered polymers (e.g., polyethylene oxides) for biomedical applications has been extensive in recent years, no consistently satisfactory molecular-level approach to drag reduction and lubrication has been developed for long-term use. Perhaps answers rest with the confounding complexity of natural fluids and surfaces at the molecular level. Preliminary experiments have shown that proteins and other macromolecules in seawater and other biological fluids can form multicomponent films that have lower coefficients of friction than films formed from the same fluids under low shear conditions. Films formed from less complex fluids do not so easily reduce interfacial friction or drag.

The report resulting from the October 1995 National Institutes of Health workshop titled "Biomaterials and Medical Implant Science: Present and Future Perspectives" outlined approximately 25 priorities for the design, development, and manufacturing of safe and effective medical devices (Watson, 1996). These priorities included:

- Biologically based materials—including "smart" materials for cell-based, drug-based, and gene-based therapies—designed by building biological structure and function into materials.
- Cross-disciplinary core infrastructures in research, design, and education.
- Development of strategies for synthesis and methods for generating new materials and coatings and development of new, alternative, or improved materials.

All these priorities present open invitations to marine scientists and marine natural products to enter the biomedical field.

There continues to be a substantial need for self-cleaning materials and lubricating coatings in the biomedical environment. Typical applications include many types of implanted devices (e.g., catheters of all types, heart valves, and contact lenses) and extracorporeal devices (e.g., dialysis membranes and blood oxygenators), as well as palliative treatment for clinical conditions, such as Sjogren's syndrome (dry eye and dry mouth).

Flotsam, Jetsam, and Neuston

In addition to harmful anthropogenic components at certain times, the sea surface is a treasure chest of organisms (including bacteria), surfactants, and proteins. Concentrations of materials in the uppermost micrometer and millimeter of the ocean's surface (MacIntyre, 1974) are addressed in terms of "enrichment factors," the ratios of microlayer concentrations to subsurface concentrations. A key function of the sea-surface layer is control of gas and chemical exchange between the atmosphere and the water (Liss and Duce, 1997). Might there be additional lessons here for biomedical applications? Can marine surfactants and proteins provide technology for improved treatment of lung failure, renal failure, or acute liver failure? Is there a biocompatible, oxygen-carrying marine material from neuston or plankton that could serve as an artificial blood? And, getting back to the eyes and mouth, can Sjogren's syndrome be overcome by a protein or surfactant from the sea surface that would lubricate and allow gas exchange and retard evaporation from natural mucosal surfaces? What are the qualities of killer whale "tear gel" in this regard?

Marine Product Development: "Assistive Technologies"

Although the focus of this workshop is on biomedical applications of natural marine products, we also should consider how these organisms and molecules will be renewably collected from marine life or mined from the sea surface, the subsurface, and the sea floor. Selection of suitable materials and coatings for sea surface or underwater processing facilities will be critical to minimize environmental impact and to maximize process efficiency. Self-cleaning and drag-reducing materials also have a key role to play as

assistive technologies in the seeding, harvesting, and development of natural marine products.

Surface-fouling periphyton and mussels, typically cited as troublesome invasive biofoulers, have high productivity and filtration capacities that offer promise for biotechnological product processing and water-quality management. Designs have been presented that use the bioadhesive potential of natural foulers for flow-through mussel filters to clear bioavailable contamination from effluents before discharge (Diggins et al., 2002). Scale-up of these designs for treatment and collection of trace marine natural products could be a practical path to harvesting of new medicinal agents.

Summary

The usual trend in fields of science and engineering is to replace precious natural products with synthetic substances. In at least one case, however, a natural marine product had no substitute: some fish oils were so effective as lubricants in jewel-bearing watches and highly sensitive gyroscopes, that *no* synthetic product was ever found to replace them. Instead, digital watches replaced the mechanical instruments and synthetic fluoropolymers were developed to contain (but not yet replace) the natural lubricants from the sea. Until we know otherwise, we must assume that the marine environment continues to hold many molecules of great potential for lubricants, self-cleaning surfaces, and molecular-exchange films. The following references provide some of the context for links between marine science and biomedical engineering.

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UNIFORM MICROPOROUS BIOMATERIALS PREPARED FROM MARINE SKELETAL PRECURSORS

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Highly interconnected microporous materials are difficult, if not impossible, to produce synthetically. With the aid of marine life-forms, we are now able to fabricate materials with desirable characteristics. Replamineform, meaning replicated life-forms, describes a method for making microporous materials made by using the calcium carbonate skeleton of several forms of marine life as a template (White et al., 1972). Three-dimensionally microporous skeletons are found in echinoderms and certain species of coral. The sizes of pores are uniform and range from 15 to 500 micrometers (μm), depending on the species.

Replicas of the microporous skeletal framework are prepared by investing the calcium carbonate skeleton with metals, ceramics, or polymers and then removing the calcium carbonate with a mild acid solution. The residual material is an interconnected porous structure. Precursor skeletons and the composite structures can be easily shaped to desired configurations prior to or after casting.

Replamineform materials have many applications; to date, they have been primarily evaluated as medical devices and prostheses. The size of the microporosity makes the process ideal for making artificial organs and implants that become ingrown with host tissues. Microporous biomaterials can be used to replace bone, blood vessels, trachea, and other damaged organs and tissues. High-surface-area membranes and piezoelectric-pyroelectric composites have also been described using this technology.

Replamineform Process

Replamineform biomaterials are fabricated using the calcium carbonate skeleton of certain forms of invertebrate marine life as a template. Echinoderms have a three-dimensional calcite lattice, topographically known as

a periodic minimal surface (Donnay and Paulson, 1969). Such surface divides spaces into two interpenetrating regions, each of which is a single, interconnected domain. The interface between the solid calcite phase and the organic material of the animal provides maximal surface area.

A similar structure is found in the aragonite skeletons of some perforate reef-building corals. The skeletal microstructure of the colonial coral *Porites*, for example, has a high degree of uniformity of pore diameter and a solid-to-void ratio of approximately one. Exceptionally high permeability is achieved because every pore in the meshwork is connected to all other pores (Weber and White, 1973).

Positive or negative replications of the microstructure can be produced using direct impregnation or lost wax casting techniques (Weber et al., 1971; Weber and White, 1973). The calcium carbonate template is removed by immersing the composite in dilute acid solution. Precursor skeletal materials are easily shaped to the desired configuration prior to acid etching. Biomedical-grade elastomeric polymers are most promising in this application.

Replacement of the porous calcium carbonate of corals (aragonite) and echinoids (calcite) by hydrothermal conversion with ammonium dibasic phosphate has been achieved (Roy and Linnehan, 1974). Virtually complete conversion to the calcium phosphate derivative (hydroxyapatite or whitlockite) with retention of the original microporous structure has been reliably accomplished.

Biomedical Applications

Replamineform biomaterials are under investigation in several applications. A brief overview of some of the medical applications is presented.

Hard Tissue Prostheses

The well-known capacity of bone tissue to regenerate has prompted considerable enthusiasm into its research. One area of interest is the interfacing of bone with prostheses to form permanent attachments without relying on adhesives. Replamineform microporous ceramics (alumina and titanium) and metals (vitallium) have been implanted up to 8 weeks in the cancellous bone in the lower extremity of the mongrel dog. New bone was found to grow into the pores of the materials and become mineralized (Chiroff et al., 1975). Dunn et al. (1979) reported favorable results using

segmental femoral prostheses. The implants were cast as a solid core of Zimalloy with a replamineform 300-500 μm porous coating. There was no evidence of infection, and 10 of 11 dogs were alive and ambulatory without difficulty at 17-19 months post-surgery.

Another area of interest to orthopedic surgeons is the regeneration of bone without permanent prostheses. Replamineform hydroxyapatite is being investigated as a long-term biodegradable bone substitute. It has been shown to provide a lattice through which bone regeneration occurs (Holmes, 1979). Hydroxyapatite implants have been evaluated in dogs as replacements in long-bone and mandibular discontinuities, as onlay bone grafts (frontal sinus and manidibular subperiosteal) and gluteal muscle implants (Roser et al., 1977). These studies demonstrated that hydroxyapatite implants can be custom-shaped intraoperatively, are well-tolerated by host tissues, provide a scaffolding for the ingrowth of bone and connective tissue, and do not induce bone regeneration when implanted in soft tissue.

Cardiovascular Materials

Successful replacement of diseased arteries and veins is a challenge to modern medicine. Small internal diameter synthetic prostheses have not functioned well for several reasons. First, no surface has been developed that is passive to the body's clotting mechanisms. Second, normal blood vessels have compliance properties (i.e., they expand with each pulsation), which are much greater than those of currently available prosthetic grafts (Kidson and Abbott, 1978). If there is a mismatch between the compliance of the prosthesis and the host's blood vessel, turbulence and stresses are generated within the graft. Finally, prosthetic vascular grafts do not have the porosity required (Harrison, 1961; Wesolowski, 1962). Porosity of prosthetics allows for incorporation of surrounding fibrous tissues on the outside of the graft and regeneration of a viable neointimal surface on the inside.

Our research program addresses each of these deficiencies. Replamineform vascular prostheses fabricated in medical-grade polyurethane become rapidly incorporated with a thin, stable, neointimal flow surface (White et al., 1976). Similar results have been generated using vascular grafts made of silicone rubber, and in fact, independent effects of pore size and biomaterial on tissue incorporation of the prostheses have been described (Hiratzka, 1979). Work in the laboratory of the investigators has revealed a greater than 90 percent patency rate for 4-mm I.D.,

6-cm-long silicone rubber prosthetics. Current work shows the efficacy of matching the compliance of the prostheses to the native artery.

Tracheal Prostheses

Tracheal obstruction as a result of malignant compression or trauma frequently requires resection and reconstruction of the involved segment. Replacement of the trachea with a prosthesis is difficult for the experimental surgeon, because this tubular organ functions in an environment continually contaminated by microbes. Thus, tissue incorporation of the prostheses may be inhibited by chronic infection. Early work demonstrated successful replacement for up to 21 months in mongrel dogs using 3-cm-long replamineform microporous tracheas (Nelson et al., 1979). Bioelectric polyurethane prostheses with a pore range of 120 to 180 μm appears to provide a favorable lattice for tissue incorporation of the prosthetic wall.

Potential Industrial Applications

High-surface-area membranes and piezoelectric-pyroelectric composites have been described using this technology. The potential applications of these materials have not been explored beyond early feasibility.

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BIOMATERIALS FOR TISSUE ENGINEERING, DRUG DELIVERY, AND OTHER MEDICALLY RELATED APPLICATIONS: THE MARINE SOURCE

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Emerging interest in the use of biomaterials derived from marine sources has led to a variety of new approaches for the treatment of disease.

Homopolymers and copolymers based on hydroxybutyrate and hydroxyvalerate are bacteria-derived aliphatic polyesters that exhibit thermoplastic properties. A number of bacteria, most notably *Alcaligenes eutrophus,* produce these materials as a carbon reserve under certain condi-

tions. As a biomaterial, they have the practical advantages of being inexpensive and readily produced through fermentation techniques. Their biocompatibility has been studied in a number of settings and is similar to more conventional polymer-based systems used clinically. Degradation of these polymers takes place through surface-erosion mechanisms; their degradation is ostensibly dependent on enzymatic mechanisms with slow hydrolytic degradation.

In tissue engineering, these polymers have been proposed for use as possible bone substitutes with Jiang et al. (2001), who described the development of new composites of poly(hydroxyalkanoates) and hydroxyapatite. In preliminary studies, these composites exhibited tensile strength and moduli comparable to cancellous bone. Other new work has centered on antibiotic delivery for the treatment of periodontal and other diseases.

Hydroxyapatite materials derived from coral can be formulated to allow the attachment and growth of cells. Such factors as macroporosity and microporosity and degree of degradability are important determinants in cellular and tissue response and clinical outcomes. Early studies by Laurencin et al. (1996) identified the ability of coralline materials to permit attachment and growth of osteoblasts with maintenance of phenotypic expression in these cells. In bone-tissue engineering, these hydroxyapatite materials have been used as parts of composites for hard-tissue regeneration. The hydroxyapatite acts as a reinforcing phase for the matrix and modulates mechanical properties while permitting the maintenance of biological response. This work has led to the development of a family of three-dimensional matrix forms for bone-tissue engineering that serve as templates for bone repair and regeneration.

Chitosan is a marine-derived polysaccharide material that has received increasing interest in biomedical applications. The material appears to exhibit little local or systemic toxicity at implantation, is sterilizable through various methods (such as autoclaving), and can be processed in a variety of distinct ways. The material demonstrates special properties, such as the formation of colloidal particles that can form complexes with macromolecules. Its ability to carry out this process in conjunction with DNA offers delivery-vehicle methods often at the nanolevel. In delivery of drugs ranging from antibiotics to various anticancer species, the polysaccharide is able to be utilized as a vehicle for delivery through oral, nasal, and parenteral routes. Chitosan has shown surprising affinity for a number of mesenchymal-derived cells, such as chondrocytes and osteoblasts, and thus may have important applications as part of tissue-engineered musculoskeletal matrix

systems. For bone and cartilage repair, work has begun to explore the development of polymer-chitosan matrices for tissue- engineering and drug-delivery applications using microsphere matrix technology previously applied to polymer-ceramic systems. It is hoped that this work will result in robust constructs that may be used in a variety of musculoskeletal environments.

In summary, a heterogeneous group of marine biomaterials (organic polymer based, ceramic based, and polysaccharide based) present important alternatives and challenges for use in biomedical applications. Collectively, their true places in drug delivery, gene therapy, and tissue engineering is yet to be determined.

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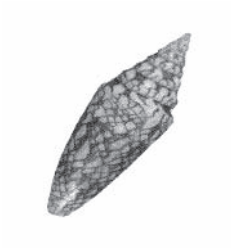

Session 4: Public Policy, Partnerships, and Outreach

BIOMEDICAL COMPOUNDS EXTRACTED FROM CORAL REEF ORGANISMS: HARVEST PRESSURE, CONSERVATION CONCERNS, AND SUSTAINABLE MANAGEMENT

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Coral reefs are among the most diverse and valuable ecosystems on earth. They provide an estimated $375 billion each year in economic and environmental services to millions of people as sources of food, construction materials, ornamentals, employment, areas of recreation and tourism, and as shoreline protection (U.S. Coral Reef Task Force, 2000). Natural products obtained from coral reef organisms in the expanding battle against human diseases and pathogenic infections are less well recognized but equally important. Benthic coral reef invertebrates contain a number of unusual, biologically active metabolites with important medical, agricultural, and industrial uses—including recent applications in bone grafting, skin-care products, and bioremediation projects—as insecticides and as potential treatments for cancer and microbial infections (Abu, 1992). Although many compounds of value have already been identified, it is estimated that less than 10% of reef biodiversity is known, and only a small

fraction of these species have been explored as a source of biomedical compounds (Fenical, 1996). New avenues for the commercial development of marine-derived compounds may further enhance the use of coral reef resources and contribute to the global economy. However, it is critical that a new paradigm is established that maximizes coral reef conservation efforts while promoting sustainable use.

Despite a significant human dependence on and concerns for coral reef ecosystems, compelling scientific evidence indicates that current human use and allocation of reef resources are threatening both the ecological and the social sustainability of these ecosystems. Increased harvest pressure is being placed on reef resources to supply subsistence fisheries as well as a growing international demand for reef species for food, traditional medicines, and ornaments. Unfortunately, few countries have sufficient knowledge, financial resources, or technical expertise to develop management plans for the sustainable harvest of reef species, and organisms are often extracted unsustainably for short-term economic gains. Although several coral reef species have yielded potential therapeutic agents, concern about adequate supply for preclinical and clinical studies is a critical issue in the development of new biomedical products. Many of the suitable reef species have a limited distribution or occur at a low biomass. Also, individuals often contain only trace amounts of the desired compounds; the low yield requires the harvest of substantial biomass, which may lead to depletion of natural populations (Creswell, 1995). Many species extinctions are predicted in the coming decades in response to increasing pressure from human activities and natural disturbances, and the pharmacological potential of coral reefs may be lost. The continued, largely unregulated, and unsustainable extraction of reef species may have consequences that extend far beyond the overexploitation of these organisms, as their removal may also affect associated species and communities, ecological processes, and even entire ecosystems that are critical to the overall health of the oceans.

To guarantee a continual source of coral reef organisms for biomedical research that can provide new medicines far into the future, resource managers need to ensure that harvest pressure does not contribute to the global decline of coral reefs. The first and foremost step to address sustainable harvest of reef species involves a shift from traditional, single-species fishery management approaches to an ecosystem approach that integrates the needs of the species, the environment, and society. Existing management approaches, which were developed primarily for food-fish species, typically involve managing individual species with little consideration of fishing im-

pacts on the rest of the ecosystem. Truly sustainable harvest will require changes in management approaches that emphasize precaution and imply a shift in focus from maximizing yield to minimizing ecological impacts and maintaining long-term biological and economic stability (Weeks and Berkeley, 2000). This shift in focus requires the consideration of all interactions of a target species with competitors, predators, and prey species; effects of the environment and natural disturbances; complex interactions between the target species and their habitat; and the effects of extraction on the species and its habitat.

An ecosystem approach to manage the collection of benthic reef species for biomedical research presents numerous challenges, as available information is inadequate on the biology, ecology, and population dynamics of most reef invertebrates. Through the development of partnerships among government agencies, commercial pharmaceutical companies, academia, and local communities, research in identification and screening of bioactive compounds can expand, with concurrent efforts directed toward sustainable management approaches. Benefit sharing with source countries can create economic incentives for reef conservation, provided that mechanisms are in place to direct revenues from bioprospecting toward the development of national and regional conservation programs (Verhoosel, 1998). Possible conservation strategies include the development of a system of marine protected areas and studies to catalogue the diversity and status of the resources contained within these areas. Basic research is also needed on the biology of the target species, linkages among coral reef organisms, and ecosystem processes controlling the distribution and abundance of target species. Finally, a greater emphasis must be placed on coral reef monitoring programs to evaluate harvest impacts and other threats, to provide information needed to establish a sustainable quota, and to adjust management measures in response to new information or subsequent disturbances.

Bioprospecting in coral reef environments offers developing countries an opportunity to derive income from the process of natural product research and development and can create economic incentives for biodiversity conservation. An ecosystem management approach will help prevent overexploitation of reef species, thereby offering a continual source of new products. However, additional strategies may be needed to reduce demand when a species is shown to contain valuable bioactive metabolites. In previous instances, up to 1 kilogram of a bioactive metabolite was necessary for clinical evaluation, and a more intensive, longer-term harvest may be required to support commercial production (Duckworth, 2001). Mass pro-

duction of the target organism through captive-breeding or mariculture may provide a consistent alternative supply without requiring sophisticated equipment for harvest or harvest techniques that are suitable for environmentally sensitive reef environments. Species in demand for the aquarium trade, live reef food-fish markets, and other seafood, and a variety of invertebrates as sources of bioactive compounds, are promising new species for intensive farming. Technology is also being applied to replenish and enhance wild stocks (Bell and Gervis, 1999). There is a growing awareness of the risks associated with mariculture (e.g., introduction of diseases or invasive species, dilution of gene pools and increased biological interactions with other species), but sustainable mariculture can be achieved with responsible application of technology and the use of indigenous species. Selective husbandry and other well-defined mariculture protocols may provide a new tool to improve the yield and quality of bioactive compounds, further reducing the number of individuals needed to provide large quantities of metabolites. Benefit sharing with source countries is a critical step that can provide the financial incentive for field research and monitoring, development of appropriate management strategies that promote sustainable use, and expanded mariculture efforts. Coral reef resources with important biomedical applications have the critical character of being renewable, at least when they are properly managed. Coral reef organisms that are abused can also become extinct, and potential medical benefits from these species will be lost forever.

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PRODUCTIVE PARTNERSHIPS IN NATURAL PRODUCT DISCOVERY AND DEVELOPMENT

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Pharmaceutical research and development projects around the world are increasingly carried out through partnerships among diverse organizations. These partnerships are frequently international and may encompass highly diverse organizations to take advantage of differential expertise, technology, access to biological materials, and arrangements for sharing of benefits.

Observations on the nature and history of the International Cooperative Biodiversity Groups (ICBG) offer useful lessons on factors that predispose partnerships to stumble or succeed. The ICBGs are multidisciplinary international consortia often involving public- and private-sector institutions in efforts to discover simultaneously new pharmaceutical and agricultural agents from natural sources, and to promote scientific and economic development and biodiversity conservation in developing countries. The non profit side of these projects is supported by cooperative agreements under a joint effort of the National Institutes of Health (NIH), the National Science Foundation, and the U.S. Department of Agriculture and is administered by the Fogarty International Center of the NIH. To date, no marine ICBGs have competed successfully for funding. However, a number of the lessons these ambitious projects have yielded are likely to be useful to marine natural products and biotechnology partnerships.

The actors in today's natural product partnerships include universities, for-profit companies, governmental agencies, conservation organizations, foundations, communities, and advocacy groups. Many partnerships among diverse organizations founder, because each entity applies its own business, cultural, or legal rules to the behavior of an entirely different type of organization. Academic scientists, for example, often mistakenly assume that the intellectual interest and good will of a collaborating industrial sci-

entist will be sufficient to maintain the commitment of that individual's company. However, pharmaceutical companies have become very dynamic in recent years. For example, in three ICBGs involving academic and industrial scientists, the collaborative efforts of the pharmaceutical companies were initiated and directed by senior scientists who had expressed personal interest and commitment to their respective group programs. But, in each of these three cases, the companies underwent mergers and subsequent major changes in their natural product research strategies within 2 years of the initiation of the projects. In all three cases, the companies decided to withdraw from these projects, along with many of their other natural products collaborations.

Similarly, companies, universities, and U.S. NGOs may run into trouble if their representatives assume that the leader of a local governmental organization or an indigenous community can speak for and sign agreements representing his or her entire constituency. Frequently, such organizations have internal clearance and consensus-building procedures that they must elaborate before embarking on a significant project, even if they are sometimes willing to try to short-circuit the process to accommodate the needs of potential partners. These internal processes are generally opaque to the outsider and often time consuming, but it is absolutely necessary to allow for them in order to develop a sustainable collaborative project.

Arrangements for the treatment of proprietary information are frequently a challenge to diverse partnerships. Host country governmental agencies may want to document species names, collection sites, and other information to enhance management of natural resources or to track collection and research efforts to protect the interests of their countries. Furthermore, academic scientists clearly need to publish their research to advance science and their own career productivity. However, conflicts often arise, because companies generally wish to prevent their competitors from seeing their assays, other research methods, and discoveries, sometimes even after patenting them. Community and conservation groups frequently also wish to keep the names, locations, and traditional uses of biological collections confidential to protect their proprietary interests or minimize overharvesting of threatened species by opportunists.

Poorly defined or overly restrictive confidentiality requirements can lead to wasted or duplicative research efforts and missed opportunities, thereby undermining the complementarity and synergy that most partnerships seek. For example, the absence of precise taxonomic information on a collection led one academic laboratory to waste an entire week of bioassay

guided isolation efforts on a species they had already worked on in the previous year. In another example, a company wasted a substantial amount of effort struggling with unidentified, nonspecific binding agents in a new class of assays. An open discussion of the matter with the field biologists would probably have led them much earlier to the identification of these agents and a means to deal with them.

Even when all the members of a partnership work well together, the partnership may stumble or fail because of external political or legal factors. For example, at least 50 countries have defined or are developing some type of legislation related to access to biological diversity and benefit sharing. However, few have implemented these laws in clearly defined normative procedures. Many of these countries are still negotiating the relationship of national sovereign rights to permit or participate in agreements on genetic resources with their own provincial governments or indigenous peoples' organizations and with super national bodies, such as the Andean Pact.

Major issues that affect the success of partnerships in addition to scientific and technical capability include an organization's stability, its administrative competence, and leadership for the project as a whole. Although exceptions exist, it is my experience that stability and predictability of research programs is highest in academia, followed by governmental programs, followed by industry. In part, this reflects the career tracks of the individuals in critical positions, but also the relative stability of the organizational types as well.

Strong and enduring partnerships are most likely to be formed by organizations that have symmetrical needs and enthusiasm for the collaboration, even if they are relatively simple arrangements limited to the exchange of specimens for technology. Hence, a partnership between a large pharmaceutical company with many sources of specimens and a small, resource-poor university in a developing country is inherently vulnerable, unless the relationship is anchored by a third organization, such as a U.S. university, that has more symmetrical relationships with the other two.

In most cases, one settles for a partnership with an organization that is less than the ideal in one or more of these respects. The organization may not have the full research capacity sought, or it may not be completely stable or administratively competent. However, the key to success is understanding its interests and capacity and planning accordingly. Conservative benchmarks, contingency plans, contract incentives, sharing of expertise and other tools can minimize the impact of these issues if one has a realistic understanding of the organization's strengths and weaknesses at the outset.

Successful and enduring partnerships in natural products and biotechnology generally have strong leadership and carefully chosen partners, and they operate in an environment of mutual respect and fairness. They most often persist over time when the partners have both strong personal and institutional commitments, and the partnership is governed by well-defined but flexible contractual arrangements and regulations.

COMMERCIALIZATION OF MARINE BIOPRODUCTS: INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER ISSUES

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The commercial development of successful pharmaceutical products and medical devices is costly, time consuming, and complex. For example, commercialization of an innovative therapeutic agent from a new chemical entity (NCE) typically requires the investment of hundreds of millions of dollars. Successful commercialization further requires the sustained, coordinated efforts of thousands of people working together, worldwide, for periods of 10 years or more toward achievement of a single goal: market entry. For biomedical technologies, commercial development encompasses a dauntingly broad array of functions, including nonclinical testing (both pharmacological and toxicological), regulatory and legal affairs, clinical research, product formulation, manufacturing, packaging, labeling, marketing, sales, education, and post-market surveillance (Cato, 1988; Trenter, 1999).

Creating a commercially successful pharmaceutical product or medical device is also risky. The transition from initial laboratory-based proof-of-concept development to early-stage commercial development is so exceedingly difficult that it is known colloquially among technology developers as "The Gap" or "The Valley of Death." In pharmaceutical development, "The Gap" is perhaps more appropriately called "The Abyss," since the vast majority of promising research-stage therapeutic agents fail to enter clinical testing. Of those investigational new drugs that enter clinical testing, only a small proportion reach the marketplace. If a pioneering new product does manage to succeed commercially, competing generic products are guar-

anteed to enter the marketplace and erode profit margins and market share as soon as the pioneering product loses exclusivity.

For those reasons, corporations and their shareholders will invest the requisite capital and commit the necessary personnel to development of a biomedical product only when effective intellectual-property protection guarantees exclusivity for that product after market entry. It is not surprising then, that intellectual-property rights form one of the cornerstones on which the modern biomedical industry is based. Intellectual-property protection is essential to the successful commercialization of marine biomedical technologies.

Intellectual property protection can take a variety of forms, including patents, trademarks, copyrights, trade secrets, and the ownership rights derived from possession of novel tangible materials (Smith and Parr, 1998). In the United States, the Drug Price Competition and Patent Term Restoration Act of 1984 established supplemental exclusivity periods that can provide patent-like protection for new medicines for up to 7 years. Patent rights constitute the most significant form of intellectual-property protection for development-stage biomedical products and are especially important for inchoate pharmaceutical products. As biomedical products move through the industrial development pipeline, however, patent-based exclusivity is often supplemented by other intellectual property rights, particularly trademarks, trade secrets, and copyrights.

When marine bioproducts are discovered at universities and nonprofit research institutes, commercialization is dependent on successful transfer of the nascent technology from its nonprofit laboratory birthplace to the industrial development pipeline. University-industry technology transfer was first envisioned in its modern embodiment by Internet prophet Vannevar Bush in 1945. Following passage of the Bayh-Dole Act in 1980, university-industry technology transfer expanded dramatically. The most recent survey conducted by the Association of University Technology Managers credited technology transfer with generating over $40 billion in product sales, $5 billion in tax revenue, and 270,000 jobs in the United States during fiscal year 1999 alone (Pressman, 2000). These numbers underscore the economic rewards that can be reaped from successful technology transfer. Less easily quantified, but much more important, are the societal benefits that flow from technology transfer in the form of new medicines and other products that improve the quality of life.

Success in technology transfer involves understanding and respecting both academic and corporate cultures, anticipating problems arising at the

interface of these two cultures, and finding creative solutions to resolve problems before they escalate into major issues. Basic research scientists in universities and nonprofit institutions can facilitate technology transfer by deepening their understanding of the breadth of the commercial development process and the role played by patents and other forms of intellectual property. A research scientist's awareness of and respect for the "development" in research and development often makes the critical difference between success and failure in technology transfer. Likewise, members of industry can enhance the effectiveness of industrial technology transfer programs by cultivating an appreciation of the resources, special challenges, and institutional constraints that exist in the workplaces of academic scientists.

The legal transfer of intellectual-property rights from university to industry is typically accomplished via a license agreement to an existing corporation that possesses the resources and motivation to bring the licensed technology into the marketplace (Smith and Parr, 1998). Transfer of the scientific and technical knowledge underlying the license can be facilitated by forming partnerships between academic research groups and corporate development teams. When an invention is potentially disruptive to the research-and-development pipeline of established corporations, technology transfer can be achieved by "spinning out" the discovery from the university into a start-up company. Such start-ups—small, nimble corporations specially formed to commercialize aggressively and creatively a new technology—play a peculiar role in U.S. innovation (Abramson et al., 1997).

For many taxonomic groups of marine organisms, diversity tends to increase with decreasing latitude. As a consequence of this biogeographic trend, the commercial development of marine bioproducts by industrialized nations involves the conversion of raw materials, harvested from developing countries, into value-added products. Furthermore, within the United States, many economically distressed coastal communities are situated in areas with rich marine bioresources. This situation brings into focus the monetary value of marine biodiversity, providing a hard-nosed economic rationale to supplement moral, ethical, and aesthetic arguments in support of marine conservation. As the United States moves to strengthen its support for marine bioproduct commercialization, an opportunity exists to earmark a portion of future financial windfalls for support of marine conservation and sustainable coastal development, thus preserving as-yet-undiscovered marine bioproducts for the benefit of future generations.

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PLANNING, PARTNERSHIPS, AND PROGRESS IN MARINE BIOTECHNOLOGY RESEARCH AND OUTREACH IN FLORIDA

James C. Cato
Director
William Seaman, Jr.
University of Florida Sea Grant

The Florida Sea Grant College Program is working to enhance both the immediate quality and future funding base for marine biotechnological research and education in Florida. Since 1996, a collegial effort has been underway to plan strategy, create partnerships, and increase funding. This effort began with the formation of a statewide faculty-industry group to advise on long-range planning. In 1997, two faculty participated in a national press briefing, a faculty-industry roundtable was held, a popular magazine style outreach document was published, and Florida faculty were successful in securing funds through national competitions. In 1998, a committee to advance Florida marine biotechnological research and education drafted a plan and assisted in or organized sessions at the state and national levels with the industry organization BIOFlorida and its national counterpart. During 2000, a second faculty-industry roundtable was held at BIOFlorida's annual meeting. During 2000 and 2001, work to create a marine biotechnological research, development, and training program was initiated in the Florida legislature, and a statewide directory of research and education faculty was published.

From a single research project in 1996, Florida Sea Grant has substan-

tially expanded its marine biotechnology research, which now represents one of its two most important areas. Fourteen projects have been completed or will be completed by 2001. Another seven projects are to begin in 2002. The emphasis is on synthesis of bioactive agents, which in turn bears on sustainability of supply of potential pharmaceuticals and industrial compounds. Several other projects focus on developing anti-fouling compounds, detecting pollutants in coastal waters, improving plants for use in dune stabilization, and identifying fish for management purposes.

A statewide faculty-industry meeting in 1996 helped to define a long-range strategic plan, a research agenda, and education and development efforts. The increased research is a direct result of this planning. A call for more outreach also resulted in the development of a statewide magazine-style report on marine biotechnology. This report has assisted in bringing more visibility to the overall effort. A faculty-industry "summit" in 1997 identified bottlenecks and actions to resolve them and produced a consensus on building statewide capabilities. This event resulted in a 1998 invited session on marine biotechnology for the statewide meeting of BIOFlorida, the new industry trade organization. Research results were presented and connections made with industry partners. A second more formal "summit" in 2000, in association with BIOFlorida, attracted at least half of Florida's faculty working in this field. Graduate students were involved, a scientific poster session was held, and a session dealt with legislation, scientific advances, and success stories from other biotechnological fields. Two marine scientists now serve on the board of directors of the industry trade organization. A statewide directory of faculty and research scientists interested in marine biotechnology research, development, and training capabilities to advance science and commerce has been completed and is available in print and on the Florida Sea Grant website.

All this activity has fostered the creation of a statewide "virtual" marine biotechnological academic department. Leadership from the strategic-planning groups and meetings organized with faculty and industry input drafted a plan to create the Florida Marine Biotechnology Research, Development, and Training Program. This plan drew the attention of the Florida legislature and the 1999-2000 and 2000-2001 sessions considered legislation to create a research, training, and development program for marine biotechnology in an academic-industry partnership. The legislation defines research priorities for the program, authorizes an appropriation, defines how proposals will be competitively selected, and creates the framework for university-industry cooperation for research projects. The bill passed all com-

mittees of the Florida house and senate during 2001 but was not passed. Attempts at passage will continue during the 2002 session of the legislature.

Statewide leadership by Florida Sea Grant since 1996 has resulted in a number of positive benefits. It has established a coherent source of funds for research and graduate students and initiated outreach to inform public audiences about marine biotechnology. It fostered the creation of a statewide network among university faculty and staff. Florida's position among states that are national leaders in marine biotechnology has been promoted. Finally, an effort to establish long-term funding for research, education, and development has been initiated, and bridges have been built for partnerships between industry and academia.

Appendixes

Appendix A

Committee and Staff Biographical Sketches

COMMITTEE CHAIR

Nancy M. Targett is a professor of marine biology-biochemistry at the Graduate College of Marine Studies at the University of Delaware. Dr. Targett earned her Ph.D. in oceanography in 1979 from the University of Maine. Her expertise is in biological oceanography and her research focuses on marine chemical ecology/organismal interactions mediated by naturally occurring metabolites, including: plant/herbivore interactions, predator/prey interactions, detoxification of allelochemicals, chemoattraction, and biofouling. She is an associate editor for the *Journal of Chemical Ecology* and an Aldo Leopold Leadership Program Fellow. From 1994-2000 she held an appointment to the Mid Atlantic Fisheries Management Council where she chaired several of their committees, and she is currently a member of the National Research Council's Ocean Studies Board.

COMMITTEE MEMBERS

Robert E. Baier received his Ph.D. in biophysics from the State University of New York (SUNY) at Buffalo (1966). Dr. Baier is a professor and director of the Industry/University Center for Biosurfaces, at SUNY Buffalo. Dr. Baier's research interests are in interrelationships of surface chemistries, biological particle adhesion, and hydrodynamic factors, as well as compli-

ant foul-release coatings. Dr. Baier served on an NRC Ocean Dumping review panel.

William H. Gerwick received his Ph.D. in oceanography from the University of California at San Diego (1981). Dr. Gerwick is a professor in the College of Pharmacy at Oregon State University. Dr. Gerwick's research interests are the bioassay-guided isolation of novel marine natural products, emphasizing those of marine microalgae, and natural product biosynthetic processes. His research broadly focuses on the exploration of marine algae as sources of new and useful biomedicinal agents.

Darrell Jay Grimes received his Ph.D. in microbiology from Colorado State University (1971). Dr. Grimes is the dean of the College of Marine Sciences at the University of Southern Mississippi. Dr. Grimes' research interests are microbiology of waste disposal and environmental contaminants. Dr. Grimes is an Ocean Studies Board member. He also served on the NRC's Committee on Climate, Ecosystems, Infectious Diseases, and Human Health.

John F. Heidelberg received his Ph.D. in marine-estuarine environmental sciences from the University of Maryland (1997). Dr. Heidelberg is an assistant investigator at the Institute for Genomic Research. Dr. Heidelberg's research interests are genomics, aquatic microbial ecology, development of 16S rRNA probes and application of molecular techniques to the study of microbial ecology.

Shirley A. Pomponi received her Ph.D. in biological oceanography from the University of Miami, RSMAS (1977). Dr. Pomponi is the Director of the Division of Biomedical Marine Research at the Harbor Branch Oceanographic Institution. Dr. Pomponi's research interests are on the systematics and cell biology of marine sponges. A major emphasis of her research is on the development of methods for sustainable use of marine resources for drug discovery and development. Dr. Pomponi was a member of the NRC's Committee on the Ocean's Role in Human Health and currently serves as vice-chair for the NRC's Committee on Exploration of the Seas.

Roger C. Prince received his Ph.D. in biochemistry from the University of Bristol, England (1974). Dr. Prince is a scientific associate at ExxonMobil Research and Engineering Company. Dr. Prince's research interests are in

understanding biological oxidation-reduction processes, especially as they relate to photosynthesis, hydrocarbon degradation, and bioprocessing. Dr. Prince served on the NRC's Committee on Opportunities for Advancement of Marine Biotechnology in the United States.

STAFF

Jennifer Merrill (project director) earned a Ph.D. in marine and estuarine environmental science from the University of Maryland Center for Environmental Science (1999). Dr. Merrill is a program officer for the NRC's Ocean Studies Board and staffs a broad range of topical studies. Her research interests include watershed and wetland management, geochemistry, and nutrient cycling in coastal systems.

Denise Greene has 7 years of experience working for the National Academies and is currently a senior project assistant for the NRC's Ocean Studies Board.

Appendix B

National Research Council Project Oversight Boards

OCEAN STUDIES BOARD

NANCY RABALAIS (*Chair*), Louisiana Universities Marine Consortium, Chauvin
ARTHUR BAGGEROER, Massachusetts Institute of Technology, Cambridge
JAMES COLEMAN, Louisiana State University, Baton Rouge
LARRY B. CROWDER, Duke University Marine Laboratory, Beaufort, North Carolina
G. BRENT DALRYMPLE, Oregon State University (ret.), Corvallis
RICHARD B. DERISO, Inter-AmericanTropical Tuna Commission, La Jolla, California
EARL DOYLE, Shell Oil (ret.), Sugar Land, Texas
ROBERT DUCE, Texas A&M University, College Station
WAYNE R. GEYER, Woods Hole Oceanographic Institution, Massachusetts
D. JAY GRIMES, University of Southern Mississippi, Ocean Springs
MIRIAM KASTNER, Scripps Institution of Oceanography, La Jolla, California
CINDY LEE, State University of New York, Stony Brook
RALPH S. LEWIS, Connecticut Geological and Natural History Survey, Hartford
BONNIE MCCAY, Rutgers University, New Brunswick, New Jersey

JULIAN P. MCCREARY, JR., University of Hawaii, Honolulu
JACQUELINE MICHEL, Research Planning, Inc., Columbia, South Carolina
RAM MOHAN, Blasland, Bouck & Lee, Inc., Annapolis, Maryland
SCOTT NIXON, University of Rhode Island, Naragansett
JON G. SUTINEN, University of Rhode Island, Kingston
NANCY TARGETT, University of Delaware, Lewes
PAUL TOBIN, Xtria, LLC Chantilly, Virginia

OCEAN STUDIES BOARD STAFF

MORGAN GOPNIK, Director
SUSAN ROBERTS, Senior Program Officer
DAN WALKER, Senior Program Officer
JOANNE BINTZ, Program Officer
JENNIFER MERRILL, Program Officer
TERRY SCHAEFER, Program Officer
JOHN DANDELSKI, Research Associate
ROBIN MORRIS, Financial Officer
SHIREL SMITH, Administrative Associate
JODI BACHIM, Senior Project Assistant
NANCY CAPUTO, Senior Project Assistant
DENISE GREENE, Senior Project Assistant
DARLA KOENIG, Senior Project Assistant
JULIE PULLEY, Project Assistant
ALISON SCHRUM, Project Assistant

BOARD ON LIFE SCIENCES

COREY GOODMAN (*Chair*), University of California, Berkeley
R. ALTA CHARO, J.D., University of Wisconsin at Madison
JOANNE CHORY, The Salk Institute for Biological Studies, La Jolla, California
DAVID GALAS, Keck Graduate Institute of Applied Life Sciences, Claremont, California
BARBARA GASTEL, Texas A&M University, College Station
JAMES GENTILE, Hope College, Holland, Michigan
LINDA GREER, Natural Resources Defense Council

ED HARLOW, Harvard Medical School, Boston, Massachusetts
ELLIOTT MEYEROWITZ, California Institute of Technology, Pasadena
ROBERT PAINE, University of Washington, Seattle
GREGORY PETSKO, Brandeis University, Waltham, Massachusetts
STUART PIMM, Columbia University, New York
JOAN ROSE, University of South Florida, St. Petersburg
GERALD RUBIN, Howard Hughes Medical Institute, Chevy Chase, Maryland
BARBARA SCHAAL, Washington University, St. Louis, Missouri
RAYMOND WHITE, DNA Sciences, Fremont, California

BOARD ON LIFE SCIENCES STAFF

FRANCES E. SHARPLES, Director
JENNIFER KUZMA, Senior Program Officer
KERRY A. BRENNER, Program Officer
JOAN G. ESNAYRA, Program Officer
MARILEE K. SHELTON, Program Officer
ROBIN A. SCHOEN, Program Officer
ROBERT YUAN, Program Officer
LAURA T. HOLLIDAY, Research Assitant
BRIDGET K.B. AVILA, Senior Project Assistant
DENISE D. GROSSHANS, Project Assistant
VALERIE L. GUTMANN, Project Assistant

Appendix C

2001 Marine Biotechnology Workshop: Biomedical Applications of Marine Natural Products

AGENDA

The National Academies
2101 Constitution Avenue, NW
Washington, D.C. 20418

MONDAY, November 5, 2001

8:00 a.m. Breakfast

8:30 a.m. Introductions and welcome —*Nancy Targett, Committee Chair, University of Delaware, Jennifer Merrill, Study Director, Ocean Studies Board*

SESSION 1: DRUG DISCOVERY AND DEVELOPMENT

8:45 a.m. Session chairs—*Shirley Pomponi, Harbor Branch Oceanographic Institute, William Gerwick, Oregon State University*

8:55 a.m. Accessing new materials: Supply issues, uncultured species—*William Fenical, Scripps Institution of Oceanography*

9:25 a.m. Discussion

9:35 a.m. Novel screening directions and technologies: Analytical techniques, retrospective views, development bottlenecks—*Guy Carter, Wyeth Ayerst*

10:05 a.m. Discussion

10:15 a.m. Break

10:30 a.m. The oceans: A rich source of drugs to treat human disease—*Mary Ann Jordan, University of California, Santa Barbara*

11:00 a.m. Discussion

11:10 a.m. Ecological roles: Mechanisms for discovery of novel targets, comparative biochemistry—*Patrick Walsh, Rosenstiel School of Marine and Atmospheric Science*

11:40 a.m. Discussion

11:50 a.m. Molecular biology and natural products—*Bradley Moore, University of Arizona*

12:20 p.m. Discussion

12:30 p.m. Lunch

SESSION 2: GENOMIC AND PROTEOMIC APPLICATIONS FOR MARINE BIOPRODUCT DISCOVERY

1:30 p.m. Session chairs—*D. Jay Grimes, University of Southern Mississippi, John Heidelberg, The Institute for Genomic Research*

1:40 p.m. The genomics revolution: Challenges and opportunities—*Claire Fraser, The Institute for Genomic Research*

2:10 p.m.	Discussion
2:20 p.m.	Bringing culture to the uncultured: Microbial discovery by high throughput cultivation—*Stephen Giovannoni, Oregon State University*
2:50 p.m.	Discussion
3:00 p.m.	Microbial microarrays: Utility, limitations and future applications, lessons learned from several model systems—*Scott Peterson, The Institute for Genomic Research*
3:30 p.m.	Discussion
3:40 p.m.	Break
4:00 p.m.	Microbial genomics: Where do we go now?—*Daniel Drell, U.S. Department of Energy*
4:30 p.m.	Discussion
4:40 p.m.	Summary discussion of events, led by Nancy Targett
5:15 p.m.	Reception – Rotunda
6:00 p.m.	Evening lecture—Marine biotechnology, past, present and future—*Rita R. Colwell, National Science Foundation*
6:45 p.m.	Discussion
7:30 p.m.	Workshop adjourns for the day

TUESDAY, November 6, 2001

8:00 a.m.	Breakfast
8:30 a.m.	Introductions—*Dr. Nancy Targett, University of Delaware*

SESSION 3: BIOMATERIALS AND BIOENGINEERING

8:45 a.m. Session chairs—*Roger Prince, ExxonMobil Research, Robert Baier, SUNY Buffalo*

8:55 a.m. Bioadhesives: Biocatalysis, post translational modification—*Christine Benedict, Geneva Pharmaceuticals, Inc.*

9:25 a.m. Discussion

9:35 a.m. Self-cleaning surfaces: Biolubricants, drag reduction—*Anne Meyer, State University of New York at Buffalo*

10:05 a.m. Discussion

10:15 a.m. Break

10:30 a.m. Uniform microporous biomaterials prepared from marine skeletal precursors—*Rodney White, UCLA Medical Center*

11:00 a.m. Discussion

11:10 a.m. Polymers for tissue engineering: Drug delivery and cellular therapy—*Cato Laurencin, Drexel University*

11:40 p.m. Discussion

12:00 p.m. Lunch

SESSION 4:
PUBLIC POLICY, PARTNERSHIPS, AND OUTREACH

12:50 p.m. Session chairs—*Nancy Targett, Committee Chair, University of Delaware, Jennifer Merrill, Ocean Studies Board*

1:00 p.m.	Biomedical compounds extracted from coral reef organisms: Harvest pressure, conservation concerns, and sustainable management—*Andrew Bruckner, NOAA Office of Protected Resources*
1:30 p.m.	Discussion
1:40 p.m.	Productive partnerships in natural products discovery and development—*Joshua Rosenthal, Fogarty Center (NIH)*
2:10 p.m.	Discussion
2:20 p.m.	Break
2:30 p.m.	Commercialization of marine bioproducts: Intellectual property and technology transfer issues—*Donald Gerhart, University of Oregon*
3:00 p.m.	Discussion
3:10 p.m.	Planning, partnerships, and progress in marine biotechnology research and outreach in Florida—*James Cato, University of Florida Sea Grant Program*
3:40 p.m.	Discussion
3:50 p.m.	Workshop wrap-up Session chairs present 10-minute summaries of the topics discussed
4:30 p.m.	Final discussion of the topic
5:30 p.m.	Workshop adjourns

Appendix D

2001 Marine Biotechnology Workshop: Biomedical Applications of Marine Natural Products

WORKSHOP PARTICIPANTS

Thomas Ahfeld	Minerals Management Service
Ron Baird	National Sea Grant College Program
Dale Baker	New York Sea Grant
Valerie Bernan	Wyeth Ayerst
Carole Bewley	National Institutes of Health
Kerry Brenner	National Research Council
Kay Briggs	Minerals Management Service
Leng Chee Chang	National Institutes of Health
Mrunal Chapekar	Advanced Technology Program, National Institute of Standards and Technology
Todd Ciche	Stanford University
Leland Ellis	U.S. Department of Agriculture
Melissa Flagg	U.S. Department of State
Scott Franklin	The Scripps Research Institute
Sylvia Galloway	National Oceanic and Atmospheric Administration
Gary Gilliland	National Institute of Standards and Technology
Deborah Gochfeld	University of Mississippi
Ximing Guo	Rutgers University
Kirk Gustafson	National Cancer Institute, National Institutes of Health
Yali Hallock	National Cancer Institute, National Institutes of Health

Mark Hamman	University of Mississippi
Russell Hill	Center for Marine Biotechnology, University of Maryland Biotechnology Institute
Channing Jones	University of North Carolina, Chapel Hill
Brendan Kelly	National Institutes of Health
Russell Kerr	Florida Atlantic University
Linda Kupfer	National Oceanic and Atmospheric Administration
Jennifer Kuzma	National Research Council
Eric Lacy	University of South Carolina
Niels Lindquist	University of North Carolina, Chapel Hill
Kristy Long	National Marine Fisheries Service, National Oceanic and Atmospheric Administration
Nicole Lopanik	University of Delaware
Hamta Madari	University of California, Santa Barbara
Dominick Mendola	CalBioMarine Technologies
Dale Nagle	University of Mississippi
David Newman	National Cancer Institute, National Institutes of Health
Gillian Nicholas	National Institutes of Health
Judith Nyquist	National Research Council
Paul Olin	California Sea Grant Program
John Paul	University of South Florida
Laurie Richardson	Florida International University
Daniel Romo	Texas A&M University
Lawrence Rouse	Louisiana State University
Fritz Schuler	National Oceanic and Atmospheric Administration
William Seaman	Florida Sea Grant College Program
Paul Sheldon	Acer Biosciences
Marc Slattery	University of Mississippi
Suzannah Sundby	Jacobson Holman PLLC
Ken Turgeon	Minerals Management Service
Jermey Weisz	University of North Carolina, Chapel Hill
Eugene White	X-ray Analytical, Inc.
Laurence Wilkinson	Center for Applied Marine Science & Technology, Virginia Institute of Marine Science
Cheryl Woodley	National Oceanic and Atmospheric Administration
Alex Xue	Acera Biosciences, Inc.
Yu-Dong Zhou	University of Mississippi

Appendix E

1999 Marine Biotechnology Workshop: Opportunities for Advancement of Environmental Marine Biotechnology

WORKSHOP PARTICIPANTS

JoAnn M. Burkholder	North Carolina State University
Linda Chrisey	Office of Naval Research
Chrys Chryssostomidis	Massachusetts Institute of Technology
John W. Costerton	Montana State University
Lori Denno	Delaware Nature Society
Richard E. Dodge	Nova Southeastern University
Jed Fuhrman	University of Southern California
Mark E. Hahn	Woods Hole Oceanographic Institution
Maryanna Henkart	National Science Foundation
Rosemarie Hinkel	University of Delaware
Diane Hite	Mississippi State University
George Hoskin	U.S. Food and Drug Administration
Jonathan Kramer	Maryland Sea Grant
Linda Kupfer	National Sea Grant College Program
Kenneth Lee	Maurice Lamontagne Institute
Leonard Levin	Electric Power Research Institute
David Manyak	Oceanix Biosciences
Judith McDowell	Woods Hole Oceanographic Institution
Irving A. Mendelssohn	Louisiana State University

Robert Menzer	U.S. Environmental Protection Agency
Marc W. Mittelman	Altra Corporation
Francois M.M. Morel	Princeton University
Aileen N.C. Morse	Marine Biotechnology Center, University of California, Santa Barbara
Ralph J. Portier	Louisiana State University
Roger C. Prince	Exxon/Mobil Research & Engineering Co.
Laurie L. Richardson	Florida International University
Michael Smolen	World Wildlife Fund
George Vermont	National Science Foundation
Cheryl Woodley	National Oceanic and Atmospheric Administration
Lily Young	Rutgers University
Raymond A. Zilinskas	Monterey Institute of International Studies